WASHINGTON STATE BOARD OF HEALTH

Notice of Public Meeting

Wednesday, August 20, 2025, 9:00 a.m. – 4:10 p.m.
Physical meeting location:
WA Department of Labor & Industries (Auditorium)
7273 Linderson Way SW
Tumwater, WA 98501-5414
Virtual meeting: ZOOM Webinar
(hyperlink provided below)
Language interpretation available

Final Agenda

Time	Agenda Item	Speaker
9:00 a.m.	Call to Order & Introductions	Patty Hayes, Board Chair
9:15 a.m.	1. Approval of Agenda—Possible Action	Patty Hayes, Board Chair
9:20 a.m.	2. Approval of June 4, 2025, Minutes – Possible Action	Patty Hayes, Board Chair
9:25 a.m.	3. Public Comment	Please note: Verbal public comment may be limited so that the Board can consider all agenda items. The Chair may limit each speaker's time based on the number of people signed up to comment.
10:10 a.m.	4. Announcements and Board Business	Michelle Davis, Board Executive Director
10:25 a.m.	5. Department of Health Update	Tao Sheng Kwan-Gett, Department of Health, Secretary's Designee
10:55 a.m.	Break	
11:10 a.m.	6. Department of Health Fluoride Science Review Update – Possible Action	Tao Sheng Kwan-Gett, Department of Health, Secretary's Designee Lauren Jenks, Department Staff Molly Dinardo, Board Staff Shay Bauman, Board Staff
12:30 p.m.	7. Federal Changes in Newborn Screening and Proposed Updates to Washington State's Candidate Condition Review Process – Possible Action	Kelly Oshiro, Vice Chair Molly Dinardo, Board Staff Megan McCrillis, Department Staff Evan Klein, Health Care Authority
12:50 p.m.	Lunch	

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Time	Agenda Item	Speaker
1:30 p.m.	8. Newborn Screening, Wilson's Disease Technical Advisory Committee (TAC) Recommendations - Possible Action	Kelly Oshiro, Vice Chair Molly Dinardo, Board Staff Megan McCrillis, Department of Health
2:15 p.m.	9. Health Impact Review Fiscal Year 2025 Update	Lindsay Herendeen, Board Staff Cait Lang-Perez, Board Staff Miranda Calmjoy, Board Staff
2:45 p.m.	10. Chapter 246-260 WAC and Chapter 246-262 WAC Water Recreation Rulemaking Update	Patty Hayes, Board Chair Ash Noble, Board Staff Joe Graham, Department of Health
3:05 p.m.	Break	
3:15 p.m.	11. State Board of Health Budget Overview	Michelle Davis, Executive Director Ashley Bell, Board Staff
3:45 p.m.	12. School Rule Project Update	Patty Hayes, Board Chair Ash Noble, Board Staff
3:55 p.m.	13. Board Member Comments and Updates	
4:10 p.m.	Adjournment	

- **To access the meeting online and to register:**
  https://us02web.zoom.us/webinar/register/WN_5nS8sAU2SHqCO2US1JhAIA
- **You can also dial in using your phone for listen-only mode:**
  Call in: +1 (253) 215-8782 (not toll-free)
  Webinar ID: 880 9469 7024
  Passcode: 682856

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Important Meeting Information to Know:

- Times are estimates only. We reserve the right to alter the order of the agenda.
- Every effort will be made to provide Spanish interpretation, American Sign Language (ASL), and/or Communication Access Real-time Transcription (CART) services. Should you need confirmation of these services, please email wsboh@sboh.wa.gov in advance of the meeting date.
- If you would like meeting materials in an alternate format or a different language, or if you are a person living with a disability and need reasonable modification, please contact the State Board of Health at (360) 236-4110 or by email wsboh@sboh.wa.gov. Please make your request as soon as possible to help us meet your needs. Some requests may take longer than two weeks to fulfill. TTY users can dial 711.

Information About Giving Verbal Public Comment at Hybrid Meetings:

- Individuals may give verbal public comments at the meeting, in-person or virtually, during the public comment period.
- The amount of time allotted to each person will depend on the number of speakers present (typically 1 to 3 minutes per person). We will first call on those who have signed up in advance.
- Sign up **by 12:00 Noon the day before a meeting** to participate in the public comment period:
    - Email the Board or
    - Register through the **Zoom webinar link. The Zoom webinar link is in the meeting agenda located on the** Meeting Information webpage**.**
    - If you are **attending the meeting in person** and did not sign up in advance, you may write your name on the sign-in sheet to provide comments if time allows.

Information About Giving Written Public Comment:

- Please visit the Board's Public Comment webpage for details.

WASHINGTON STATE BOARD OF HEALTH

Aviso de reunión pública

Miércoles 20 de agosto de 2025, de 9:00 a.m. a 4:10 p.m.
Lugar de la reunión presencial:
Departamento de Trabajo e Industrias de Washington (Auditorio)
7273 Linderson Way SW
Tumwater, WA 98501-5414
Reunión virtual: seminario web por ZOOM (enlace a continuación)
Servicios de interpretación lingüística disponibles

Orden del día final

Hora	Punto del orden del día	Orador/a
9:00 a.m.	Apertura y presentaciones	Patty Hayes, presidenta de la Mesa Directiva
9:15 a.m.	1. Aprobación del orden del día – Posible acción	Patty Hayes, presidenta de la Mesa Directiva
9:20 a.m.	2. Aprobación de las actas del 4 de junio de 2025 – Posible acción	Patty Hayes, presidenta de la Mesa Directiva
9:25 a.m.	3. Comentarios públicos	Aclaración: Es posible que los comentarios verbales del público se limiten para que la Mesa Directiva pueda abordar todos los puntos del orden del día. La presidenta podrá limitar el tiempo de cada orador en función de la cantidad de personas que se hayan inscrito para hacer comentarios.
10:10 a.m.	4. Anuncios y asuntos de la Mesa Directiva	Michelle Davis, directora ejecutiva de la Mesa Directiva
10:25 a.m.	5. Actualización del Departamento de Salud	Tao Sheng Kwan-Gett, delegado de la secretaría del Departamento de Salud
10:55 a.m.	Receso	
11:10 a.m.	6. Actualización de la revisión científica sobre fluoruros del Departamento de Salud – Posible acción	Tao Sheng Kwan-Gett, delegado de la secretaría del Departamento de Salud Lauren Jenks, miembro del personal del Departamento Molly Dinardo, miembro del personal de la Mesa Directiva Shay Bauman, miembro del personal de la Mesa Directiva

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Hora	Punto del orden del día	Orador/a
12:30 p.m.	7. Cambios federales en la Evaluación del recién nacido y actualizaciones propuestas para el proceso de revisión del estado de los candidatos del Estado de Washington – Posible acción	Kelly Oshiro, vicepresidenta de la Mesa Directiva Molly Dinardo, miembro del personal de la Mesa Directiva Megan McCrillis, personal del Departamento Evan Klein, Autoridad Sanitaria
12:50 p.m.	Almuerzo	
1:30 p.m.	8. Evaluación del recién nacido, recomendaciones del TAC (por su sigla en inglés, Comité de Asesoramiento Técnico) enfermedad de Wilson – Posible acción	Kelly Oshiro, vicepresidenta de la Mesa Directiva Molly Dinardo, miembro del personal de la Mesa Directiva Megan McCrillis, Departamento de Salud
2:15 p.m.	9. Actualización de la Revisión del Impacto en la Salud para el año fiscal 2025	Lindsay Herendeen, miembro del personal de la Mesa Directiva Cait Lang-Perez, miembro del personal de la Mesa Directiva Miranda Calmjoy, miembro del personal de la Mesa Directiva
2:45 p.m.	10. Capítulo 246-260 del WAC (por su sigla en inglés, Código Administrativo de Washington) y Capítulo 246-262 del WAC Actualización de la normativa sobre actividades de recreación acuática	Patty Hayes, presidenta de la Mesa Directiva Ash Noble, miembro del personal de la Mesa Directiva Joe Graham, Departamento de Salud
3:05 p.m.	Receso	
3:15 p.m.	11. Resumen presupuestario de la Mesa Directiva de Salud del Estado	Michelle Davis, directora ejecutiva Ashley Bell, miembro del personal de la Mesa Directiva

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Hora	Punto del orden del día	Orador/a
3:45 p.m.	12. Actualización del proyecto sobre normas escolares	Patty Hayes, presidenta de la Mesa Directiva Ash Noble, miembro del personal de la Mesa Directiva
3:55 p.m.	13. Comentarios y actualizaciones de los miembros de la Mesa Directiva	
4:10 p.m.	Levantamiento de la sesión	

- **Para acceder a la reunión en línea y registrarse:** https://us02web.zoom.us/webinar/register/WN_5nS8sAU2SHqCO2US1JhAIA

- **También puede participar por teléfono, mediante la modalidad de solo escucha:**
  Llamada: +1 (253) 215-8782 (no es un número gratuito)
  Id. del seminario web: 880 9469 7024
  Contraseña: 682856

Información importante de la reunión que debe saber:

- Los horarios son estimativos. Nos reservamos el derecho de modificar el orden de los puntos que se tratarán en la reunión.
- Se hará todo lo posible por brindar servicios de interpretación al español, ASL (por su sigla en inglés, lenguaje de señas americano) o CART (por su sigla en inglés, servicios de transcripción simultánea). Si necesita la confirmación de estos servicios, envíe un correo electrónico a wsboh@sboh.wa.gov antes de la fecha de la reunión.
- Si desea acceder a los materiales de la reunión en un formato alternativo o en otro idioma, o si tiene una discapacidad y necesita una modificación razonable, comuníquese con la Mesa Directiva de Salud llamando al (360) 236-4110 o enviando un correo electrónico a wsboh@sboh.wa.gov. Le pedimos que presente su solicitud lo antes posible para ayudarnos a satisfacer sus necesidades. Es posible que algunas solicitudes tarden más de dos semanas en atenderse. Los usuarios de TTY pueden marcar el número 711.

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Información para hacer comentarios públicos verbales en las reuniones híbridas:

- Las personas pueden hacer comentarios públicos verbales en la reunión, en persona o de forma virtual, durante el período de comentarios públicos.
- La cantidad de tiempo otorgada a cada persona dependerá de la cantidad de personas presentes que deseen hablar (suelen ser de 1 a 3 minutos por persona). Primero llamaremos a quienes se hayan anotado con anticipación.
- Regístrese **antes de las 12:00 del mediodía el día anterior a la reunión** para participar en el período de comentarios públicos:
    - Envíe un correo electrónico a la Mesa Directiva o
    - Regístrese a través del **enlace al seminario web por Zoom. El enlace al seminario web por Zoom está en el orden del día que figura en la** página web de información de la reunión.
    - Si va a **asistir a la reunión en persona** y no se registra con anticipación, puede escribir su nombre en la hoja de inscripción para poder hacer comentarios si el tiempo lo permite.

Información para hacer comentarios públicos escritos:

- Visite la página web de comentarios públicos de la Mesa Directiva para obtener detalles.

WASHINGTON STATE BOARD OF HEALTH

Draft Minutes of the State Board of Health
June 4, 2025
Hybrid Meeting
ASL and Spanish interpretation available
Virtual meeting: ZOOM Webinar
Physical location: Washington State Department of Health
111 Israel Road S.E., Tumwater, WA 98501
Building: Town Center Two (TC2, Rooms 166 & 167)

State Board of Health Members present:

Patty Hayes, RN, MSN, Chair
Kelly Oshiro, JD, Vice Chair
Stephen Kutz, BSN, MPH
Tao Sheng Kwan-Gett, MD, MPH, Secretary's Designee
Paj Nandi, MPH
Peter Browning, MA
Mindy Flores, MHCM

State Board of Health Members absent:

Socia Love, MD

State Board of Health staff present:

Michelle Davis, Executive Director
Lilia Lopez, Assistant Attorney General
Melanie Hisaw, Executive Assistant
Ashley Bell, Deputy Director
Michelle Larson, Communications Manager
Anna Burns, Communications Consultant
Marcus DeHart, Communications Consultant
Molly Dinardo, Health Policy Advisor
Ash Noble, Health Policy Advisor
Hannah Haag, Community Engagement Coordinator
Crystal Ogle, Administrative Assistant
Nina Helpling, School Rules Project Policy Advisor
Mary Baechler, School Rules Project Community Engagement Coordinator
Kelly Kramer, Newborn Screening Project Policy Advisor

Guests and other participants:

Lauren Jenks, Department of Health
Michael Ellsworth, Department of Health
Kelly Cooper, Department of Health
Kseniya Efremova, Department of Health
Annie Hetzel, Office of the Superintendent of Public Instruction
Lisa Mancl, University of Washington
Mike McNickle, Director, Grays Harbor County Public Health

Patty Hayes, Board Chair, called the public meeting to order at 9:01 a.m. and read from a prepared statement (on file). Michelle Davis, Board Executive Director, provided a Land Acknowledgement.

1. **APPROVAL OF AGENDA**
   Motion: Approve June 4, 2025, agenda
   Motion/Second: Member Kutz/Member Flores. Approved unanimously

2. **ADOPTION OF APRIL 9, 2025 MEETING MINUTES**
   Motion: Approve the April 9, 2025, minutes
   Motion/Second: Member Browning/Member Kutz. Approved unanimously

3. **PUBLIC COMMENT**
   Patty Hayes, Board Chair, opened the meeting for public comment and read from a prepared statement (on file).

   Bill Osmunson discussed opposing community water fluoridation for the last twenty years. B. Osmunson encouraged people to look at historical data, research, and studies. B. Osmunson said that children are dying due to fluoridated water.

   Bob Runnells referenced recent changes on the Health and Human Services (HHS) website for COVID-19 vaccines for children and pregnant women. B. Runnells said the COVID-19 vaccination page on the Department of Health website, updated May 29, does not correlate with the current HHS recommendation, and is looking for clarity.

   Derek Kemppainen discussed fluoride being a poison and shared reasons to oppose water fluoridation, comparing it to the Nuremberg Trials, illegal medical experiments, and violation of human rights.

   Mary Long spoke in opposition to community water fluoridation, and the emerging science challenges the safety and efficacy of this practice. M. Long discussed the harmful effects to children, such as the rise in dental fluorosis and skeletal concerns. M. Long suggested the Board follow Utah and Florida's lead.

   Mariah Kunz spoke in support of community water fluoridation and said it reduces health inequities, reduces dental decay, and has no evidence of harm. M. Kunz referenced a study from the Journal of the American Medical Association that additional cavities would cost billions of dollars and investing in prevention early on saves community members on costs and other chronic diseases. M. Kunz said many organizations firmly support this community health practice such as the American Academy of Pediatrics, American Dental Association, American Medical Association, and the World Health Organization.

   Laurie Layne spoke in support of the repeal of the Environmental Health and Safety Rule for schools. L. Layne said that the public is taxed enough. More programs and increased taxes are not sustainable for citizens.

   Lisa Templeton discussed the MRNA vaccine, COVID-19 studies, and the lack of safety data. L. Templeton said the public deserves full disclosure and that the data confirm

myocarditis risk. L. Templeton urged the Board to insist on rigorous trials and oppose promotion of the vaccine(s).

Grace Yuan discussed the School Rule Project (SRP). G. Yuan said that Chair Hayes and the diverse technical advisory committee membership did an incredible job. G. Yuan asked to keep the phases separate moving forward and that the fiscal analysis needs to be updated and more robust. G. Yuan said the Legislature needs to prioritize and fund the SRP and looks forward to working together.

Natalie Chavez said the measles update at the April 10 Vaccine Advisory Committee meeting regarding the death of two children was disturbing, offensive, and disrespectful. L. Templeton said no one should discuss the deaths of children without thoroughly reviewing the records. N. Chavez said the children reported had numerous risk factors, such as hospital pneumonia, E. coli bacteria in the lungs, lack of proper antibiotics, and were very sick. N. Chavez expressed concerns about the misreports and the measles death narrative.

4. BOARD ANNOUNCEMENTS AND OTHER BUSINESS

Michelle Davis, Board Executive Director, provided updates on staff and Board activities. Executive Director Davis thanked staff for their work on two year-long, legislatively funded projects, the School Rule Project and the Newborn Screening Project. Both projects will end on July 1.

Executive Director Davis shared that the Board signed onto a Congressional letter of support regarding potential impacts of the proposed federal budget on Medicaid and food access programs. Executive Director Davis mentioned upcoming meetings related to the 2026 State Health Report, Newborn Screening on Wilson Disease, and the Washington State Association of Local Public Health Officials annual meeting.

Executive Director Davis discussed the work of the Foundational Public Health Services (FPHS) Steering Committee and how reductions to FPHS funding may impact the state's governmental public health system. Ashley Bell, Board staff, and Executive Director Davis will share more about FPHS spending and discuss the Board's budget outlook for the upcoming biennium at the August 20 Board meeting. The Board's budget will be tighter and may limit the number of projects the Board can take on.

Executive Director Davis provided an update on the Health Impact Review (HIR) team. The HIR team completed seven HIRs this legislative session. The HIR team is currently working on an HIR request from Representative Thomas for Substitute House Bill 1816, concerning civilian-staffed crisis response teams. The HIR team will provide its annual update at a Board meeting this fall.

Executive Director Davis shared an update about the Governor's Interagency Council on Health Disparities (Council). There are two vacancies on the Council, including a vacancy for a representative from the Department of Health (Department) and a vacancy for a Health Care Consumer Representative. In 2024, the Legislature approved a funding increase for the Council for the first time since the Council was created in 2006. In this year's budget, the 2024 funding increase was cut in half, which is a significant amount of funding for the Council. State law requires the Board to assist the

Governor in convening the Council. Executive Director Davis stated the Board will receive an update about Council funding at the August meeting as well.

To support the Council's May 20 meeting, staff developed a baseline overview of social drivers of health and conditions in Washington State. Executive Director Davis will share a link to the report, as it may provide a helpful backdrop to the Board's work on the State Health Report.

5. DEPARTMENT OF HEALTH FLUORIDE SCIENCE REVIEW UPDATE

Kelly Oshiro, Vice Chair, provided background on the Department of Health (Department) Science Review Panel (Panel) and the Board's work on this topic. Vice Chair Oshiro noted that the Panel is not yet ready to provide recommendations to the Board and would instead provide a status update on their work. As such, no Board action or public comment would be taken on this agenda item.

Lauren Jenks, Department of Health, Assistant Secretary for Environmental Public Health, gave an overview of the Department Science Review Panel, co-chaired with Secretary's Designee Tao Sheng Kwan-Gett. The Panel, which includes Board staff, was created to review scientific evidence on community water fluoridation to help inform Board policy, Department initiatives, and public messaging. Between January and June 2025, the Panel met ten times and reviewed recent studies, including the 2024 National Toxicology Program monograph, Cochrane review, Environmental Protection Agency court judgment, and research on fluoride's health effects and benefits.

Lauren shared that the Panel discussed both the benefits of fluoridation, especially for children's oral health, and potential risks, such as possible links between high fluoride exposure and lower IQ. Lauren noted the issue remains controversial, rooted more in values than science, and that scientific uncertainty remains around health equity and neurodevelopment. The Panel is drafting recommendations for the Board, which will be reviewed by the Secretary of Health, the Governor's Office, and partners before being shared at the August 20 Board meeting (see presentation on file).

Peter Browning, Board Member, noted the challenge of isolating confounding variables in decisions about community water fluoridation and shared an example from Anacortes, Washington. Member Browning emphasized that reaching consensus and issuing a recommendation may be difficult and reminded the Board that it does not have the authority to mandate fluoridation.

Lauren shared that the Panel had also discussed confounding variables. Lauren noted that the evidence supporting the benefits of community water fluoridation was clearer before fluoride toothpaste became widely used in 1970. While earlier research demonstrated its effectiveness, current analysis must consider whether the additional benefit today justifies potential risks.

Member Browning added that this evolving context may require conversations with parents about whether to provide fluoridated drinking water for their children.

Paj Nandi, Board Member, thanked the Panel for its thoughtful and thorough approach and acknowledged the complexity of its work. Member Nandi asked what the Panel is

hearing from other states. For example, some states have already moved on to policy recommendations. Member Nandi asked if the Panel is hearing from colleagues in other states since this topic will likely become a national discussion, which may impact recommendations and understandings in Washington State.

Lauren responded that the staff are in communication with colleagues in other states. Lauren noted that while some states are experiencing statewide debates on the issue, Washington's context is more localized, with varying community perspectives and values. As a result, Washington will need to support local jurisdictions in making their own decisions, and its approach may differ from those of other states.

Steve Kutz, Board Member, provided comment on fluoride in bottled drinking water.

Lauren stated that the Panel has not discussed bottled water, but that the Department will likely need to provide specific recommendations for reconstituting infant formula.

Patty Hayes, Board Chair, expressed appreciation for the Panel's balanced and clear discussion. Chair Hayes acknowledged the ongoing challenge of aligning emerging science with policy and emphasized the importance of highlighting issues the Board should consider related to community water fluoridation. Chair Hayes then requested that before the August 20 Board meeting, Lauren provide an overview of relevant federal and state legal authorities for Board Members to review, have the Panel comment on the Board's 2015 Oral Health Recommendations, and review public comments received by the Board on community water fluoridation.

Lauren confirmed that the Panel has received the written public comments, will add the 2015 Oral Health Recommendations to an upcoming meeting agenda, and plans to address legal authority during the August 20 presentation.

Mindy Flores, Board Member, stated that the Board is looking forward to hearing the Panel's recommendations in August.

Molly Dinardo, Board staff, provided a high-level overview of federal and state authorities related to community water fluoridation. Molly explained that the federal government is responsible for evaluating the safety of fluoridation and issuing public health recommendations. But the federal government does not regulate fluoridation in water systems beyond the scope of the Safe Drinking Water Act. Molly then outlined the specific roles that the U.S. Food and Drug Administration (FDA), the Centers for Disease Control (CDC), and the U.S. Environmental Protection Agency (EPA) play in community water fluoridation. Molly noted that the only enforceable standards are through the EPA, which sets a maximum contaminant level (MCL) for fluoridate at 4.0 mg/L and a non-enforceable secondary level of 2.0 mg/L.

Molly discussed state authority related to community water fluoridation and noted that in Washington State, decisions about community water fluoridation are made at the local level by local governments or water systems. Molly emphasized that while state law establishes standards for safe drinking water, the Board does not require or prohibit fluoridation. Molly stated that for systems that choose to fluoridate, state regulations require fluoride levels of 0.7 mg/L, consistent with the U.S. Public Health Service

(PHS)/CDC recommendations. This standard was last updated by the Board in 2016. Washington's drinking water regulations also align with the EPA's maximum contaminant level (MCL) for fluoride.

Molly also provided a brief overview of the Board's 2015 Oral Health Recommendations, which were developed through a series of workshops aimed at improving oral health outcomes in Washington State. Molly noted that the recommendations are available on the Board's website. The recommendations cover a range of topics, including community water fluoridation, access to dental sealants for school-aged children, provider scope of practice, oral health literacy, statewide monitoring, and workforce development to better serve underserved communities.

Molly added that these recommendations were not part of a rulemaking process, as they fall outside the Board's regulatory authority. The recommendations have not been reviewed or updated since 2015.

Member Kutz asked whether any water systems in Washington have naturally occurring fluoride levels that exceed drinking water standards. What actions can be taken locally?

Lauren responded that some areas, primarily in Central and Southeastern Washington, do have naturally occurring fluoride levels above the EPA's MCL. In those areas, public water systems remove fluoride to stay below the standard, generally reducing levels to below 1.5 mg/L. Private well owners may test their water and consider filtration if necessary.

Member Kutz asked what types of filters remove fluoride if a homeowner would like to do that.

Tao Sheng Kwan-Gett, Secretary's Designee, stated homeowners would need to use a reverse osmosis type of filter. Member Kwan-Gett also clarified that the Panel was not evaluating whether fluoride is inherently toxic but rather focused on whether there is a safe level at which the benefits outweigh the risks. Member Kwan-Gett emphasized that in toxicology, "the dose makes the poison," and the Panel's goal is to interpret the science, acknowledging both its strengths and uncertainties, in plain language to support informed, community-based decisions.

6. **UPDATE FROM THE DEPARTMENT OF HEALTH – DELEGATED PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) EXCEPTION RULEMAKING**
   Paj, Nandi, Board Member, introduced the topic and asked Board and Department of Health (Department) staff to provide an update on delegated rulemaking for per- and polyfluoroalkyl substances (PFAS).

   Brad Burnham, Department staff, reviewed the history of the Environmental Protection Agency's (EPA) PFAS rulemaking for required monitoring, reporting, maximum contaminant levels, and public notification. Brad shared that at the May 28 Department hearing, there was no public testimony and only two written comments. The Department anticipates filing the CR-103 by June 11, 2025, with an effective date 31 days later.

The Board took a break at 10:47 a.m. and reconvened at 11:01 a.m.

7. **PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) EMERGENCY RULE, CHAPTER 246-290-315 WAC**

Paj Nandi, Board Member, introduced the topic and asked Board staff to present an update on per- and polyfluoroalkyl substances (PFAS) rulemakings and a recommendation to update the PFAS emergency rule. Department of Health (Department) staff Brad Burnham and Lauren Jenks were available for questions.

Ash Noble, Board staff, provided an overview of PFAS chemicals and past emergency rulemakings to implement federal Environmental Protection Agency (EPA) PFAS rules adopted in April 2024. Ash noted the current emergency rule expires on June 19, 2025, and requested Board approval of a fourth emergency rule. This would continue to amend Chapter 246-290-315 WAC so that the criteria of EPA's new regulations would apply on the effective date of the maximum contaminant levels (MCLs) as set in the federal standard, not the adoption date for another 120 days. Ash also stated that a CR-101 was filed in September 2024 to make permanent rule changes and expects that the CR-102 will be filed in September 2025 (see presentation on file).

Steve Kutz, Board Member, asked if the current rules contain requirements for MCLs that are stricter than what the EPA is proposing.

Brad Burnham, Department staff, explained that while the state adopted State Action Levels (SALs), the new EPA MCLs are more stringent.

Lauren Jenks, Department staff, Assistant Secretary for Environmental Public Health, said that Chapter 246-290-315 WAC currently states that more stringent federal rules take effect upon adoption rather than their effective date. This removes the Department's requirement for water systems to notify customers of PFAS exceedances. The emergency rule updates that language.

Kelly Oshiro, Board Vice Chair, asked if the Board can expect another emergency rule request in October and when the permanent rule might move forward.

Ash stated that another emergency rule request will likely happen in October and hopes to have the CR-102 filed for the permanent rule in September and expects to hold a hearing in November.

Motion: Moves that the Board direct staff to file a CR 103E to initiate emergency rulemaking for WAC 246-290-315 to continue to clearly maintain the associated requirements until the federal standards are effective.
Motion/Second: Member Kutz/Member Browning. Approved unanimously

8. **APPROVAL OF THE DRAFT REPORT ON BRANCHED-CHAIN KETOACID DEHYDROGENASE KINASE (BCKDK)**

Kelly Oshiro, Vice Chair, provided background information on this condition review.

Kelly Kramer, Board staff, summarized the report and shared that the Newborn Screening (NBS) Technical Advisory Committee (TAC) recommended not adding Branched-Chain Ketoacid Dehydrogenase Kinase (BCKDK) deficiency to the NBS

panel. Staff will send the report to the Office of Financial Management for review. Kelly asked for the Board's approval of the draft.

Peter Browning, Board Member, noted the potential for the condition to be passed on in families with a history of it. Member Browning asked whether there are efforts to educate families and recommended testing for those with a known history.

Kelly responded Yes.

Patty Hayes, Board Chair, clarified that the Department of Health (Department) doesn't have a genetic testing program. The recommendations regarding testing rely on the connection between the provider and parent.

Kelly affirmed Chair Hayes's clarification and reiterated that any testing would be between the patient and provider. The Board and the Department do not have a role in that.

Chair Hayes asked whether the report clearly states that testing for BCKDK deficiency should not be included in the newborn screening panel and instead be a decision made by the provider and parent.

Kelly responded that the team will review the report's language to ensure that it is clear. Kelly noted BCKDK deficiency is rare, with no identified cases in the U.S., and limited data makes it difficult to recommend screening at this time.

Chair Hayes clarified that they want the report to be clear and to emphasize the main point. This is an issue between the provider and the family.

Vice Chair Oshiro suggested including a sentence from page four of the report in the executive summary to highlight how there are only 21 cases worldwide.

Motion: The Board approves the BCKDK deficiency legislative report and directs staff to finalize the report in consultation with the Chair and submit it to the Governor and appropriate legislative committees by June 30, 2025. The Board directs staff to send copies of the final report to TAC Members.
Motion/Second: Member Kutz/Member Browning. Approved unanimously

9. LOCAL HEALTH AND COMMUNITY FOCUS

Kelly Oshiro, Vice Chair, gave a brief overview of Grays Harbor County, including its population, school districts, and economic landscape. Vice Chair Oshiro then introduced Mike McNickle, Director of Grays Harbor County Public Health (GHCPH) (see presentation and biography on file).

Director Mike McNickle gave an overview of GHCPH's recent efforts and challenges. After the pandemic, GHCPH prioritized a community health assessment and identified behavioral health and substance misuse as key concerns. A behavioral health gaps analysis led to ongoing work on a crisis triage system.

GHCPH launched several programs, including youth “third spaces,” mobile medical services, school-based health centers, diabetes screenings and education, a Homeless Housing Program, and expanded childcare support. GHCPH also partnered with Pacific Medical Center, restarted in-person WIC, and added environmental health services and a diaper bank.

Ongoing challenges include provider shortages, transportation barriers, lack of culturally appropriate care, affordable housing needs, and limited indoor recreation during winter. GHCPH is exploring crisis care models like EmPATH units and behavioral urgent care (see presentation on file).

Patty Hayes, Chair, complimented Director McNickle’s work and the emphasis on public health’s role. Chair Hayes expressed support for school-based health centers and likes the mobile medical van being local. Chair Hayes asked for Director McNickle’s perspective on the role between public health and the local community health. GHCPH work could be a wonderful example of how to rebuild trust in the community. Chair Hayes also mentioned that many of the GHCPH programs and activities make public health visible in a way that meets what the community has stated that it needs.

Director McNickle responded that rebuilding trust after the COVID-19 pandemic is a key goal, especially given the changing recommendations early in the pandemic. GHCPH now focuses on community priorities and works closely with partners, including Tribal representatives, to ensure broad community involvement.

Paj Nandi, Board Member, complimented the presentation and expressed interest in connecting offline about the Health Care Authority (HCA) efforts in addressing opioids in Tribal and Native communities. Member Nandi mentioned past collaboration with Grays Harbor leadership and asked what GHCPH's approach to influencing county-wide policies.

Director McNickle responded that while “Health in All Things” is ideal, “Health in Most Things” is more realistic. GHCPH holds convenings at the health summit, discusses social determinants of health, and involves providers. The goal is to improve service availability and remain persistent, recognizing that without good health, everything else suffers, especially housing and transportation.

Mindy Flores, Board Member, commended the growth of GHCPH. Member Flores asked about health in all things and whether the county has a dental care plan.

Director McNickle said that dental issues are expected to grow after one of Grays Harbor County's systems stopped fluoridating its water, especially given the limited providers. Children ages two to five already have low dental care rates. The county is using ads, social media, and the Access to Baby and Child Dentistry program to raise awareness and connect families to care. Some Federal Public Health Services (FPHS) funds were used for dental care provision; hopefully the funding continues despite upcoming cuts.

Tao Sheng Kwan-Gett, Secretary’s Designee, complimented Director McNickle’s work.

Peter Browning, Board Member, complimented Director McNickle's energy and suggested exploring partnerships with transit authorities for low-cost transportation options. Member Browning noted that this is how Skagit County recently acquired vehicles.

Steve Kutz, Board Member, thanked Director McNickle for their presentation and asked whether fentanyl misuse is a big issue in Grays Harbor County.

Director McNickle responded that Grays Harbor County has one of the highest fentanyl use and overdose rates in the state. The county's wellness center serves about 250 clients and is expanding due to rapid growth. Director McNickle noted the need for upstream preventive strategies, like the youth third places strategy. The county has just received $1 million grant from the federal government to do opioid programming in jails.

Member Kutz thanked Director McNickle for the response.

10. 2025 SCHEDULE UPDATE – CANCEL JULY 9 BOARD MEETING

Michelle Davis, Board Executive Director, presented an update to the 2025 meeting schedule and recommended canceling the July 9, 2025, Board meeting.

Motion: The Board approves canceling the July 9, 2025, Board Meeting.
Motion/Second: Member Kutz/Member Browning. Approved unanimously

The Board recessed for lunch at 12:30 p.m. and reconvened at 1:30 p.m.

11. RULES HEARING, AUDITORY SCREENING, CHAPTER 246-760 WAC

Kelly Oshiro, Vice Chair, provided an overview of the Board's authority to adopt rules related to auditory screening and gave background on the rulemaking project.

Molly Dinardo, Board staff, and Annie Hetzel, Office of the Superintendent of Public Instruction, introduced themselves to the Board.

Molly reviewed the rulemaking process, timeline, and engagement work. Molly presented staff-recommended changes based on public comments received during the formal comment period, focusing on clarifying language and ensuring feasibility for schools and families (see presentation on file).

Vice Chair Oshiro announced the public hearing, directed attendees to related materials on the Board's website, read a statement with hearing instructions, and formally opened the hearing for public testimony. Each speaker was allotted three minutes. Testimony was accepted in person and via Zoom.

Michelle Larson, Board staff, called on public commenters who registered for verbal testimony via Zoom. Of the thirteen registered, only one was present and provided testimony.

Laurie Layne, public commenter, expressed some support for the proposed rule changes but urged caution due to taxpayers' limited budgets. L. Layne recommended making the school hearing screening programs optional to accommodate financial

constraints. L. Layne also suggested involving doctors, such as ear, nose, and throat specialists, to conduct the screenings instead of schools.

Vice Chair Oshiro closed the public testimony portion of the rules hearing and asked if Board Members had any questions.

Tao Sheng Kwan-Gett, Secretary's Designee, thanked the presenters and expressed support for the proposed revisions. Member Kwan-Gett then requested clarification on the proposed language regarding the use of otoacoustic emission (OAE) technology in the rule. Member Kwan-Gett noted their understanding that pure tone audiometry is the preferred gold standard, while OAEs are used for students unable to complete pure tone testing.

Molly directed Board Members to page 222 of the meeting materials and read the proposed addition to WAC 246-760-030 regarding required and alternative hearing screening tools. Molly explained that the rule allows the use of OAE testing for students unable to participate in pure tone screening, including those with special health care needs, developmental delays or disabilities, non-English speakers, and younger students or those who have difficulty understanding instructions. Molly added that the rule also clarifies that OAE testing is not intended to replace pure tone screening except in these specified cases.

Vice Chair Oshiro asked about the accuracy of OAE devices relative to pure tone audiometry.

Molly responded that studies comparing pure tone audiometry and otoacoustic OAE screening, mostly in clinical settings, suggest pure tone may be slightly more sensitive. However, no recent studies indicate significant differences in accuracy rates. Molly mentioned that the most recent study comparing the use of these devices in a community setting was from 2015. Molly added that both the American Academy of Audiology and the American Speech-Language-Hearing Association support the use of OAE as outlined in the proposed rules.

Michelle Davis, Executive Director, reminded Board Members that while schools may use OAE testing, it is not required—pure tone screening remains the standard. Executive Director Davis emphasized that school screening programs have been in place for decades. Addressing concerns about testing in physicians' offices, Executive Director Davis noted that school programs provide universal screening, especially for children who may lack regular access to healthcare or a medical home. Executive Director Davis noted that this context may not have been fully covered in the materials, but it is important given the public comments received.

Molly thanked Executive Director Davis for these comments and highlighted the significant legislative rule analysis (cost-benefit analysis) on page 251, required under state law for significant rules. Molly shared that the analysis concluded that the benefits of the proposed updates outweigh the costs and noted no anticipated additional costs to schools, since hearing screenings are already mandated and the new technology is optional.

Steve Kutz, Board Member, commented that this screening is the most basic thing that can happen to help kids who are struggling and may not know why. Member Kutz shared their own experience with vision and hearing screenings in school.

Patty Hayes, Board Chair, thanked the public commenter for addressing the cost concerns of rule implementation. Chair Hayes noted that the rule provides flexibility and responds to the petition from the Lake Chelan Lion's Club. Citing the clear cost-benefit analysis, Chair Hayes expressed confidence that the system can effectively integrate the new technology to support children and youth within existing budgets. Chair Hayes voiced support for the rule and its amendments.

Member Kwan-Gett agreed with Chair Hayes and shared from their primary care pediatrics experience that OAE devices frequently malfunction and incur costly maintenance. They recommended schools consider these reliability and upkeep challenges, noting that pure tone audiometry equipment tends to be more dependable.

Vice Chair Oshiro asked Molly if staff had reconnected with the petitioner during the rulemaking process.

Molly confirmed that staff did reconnect with the petitioner. Molly shared that while the petitioner appreciated the inclusion of OAE devices, they had hoped for OAE to be used as the primary screening tool. However, pure tone screening remains the standard across national screening guidelines. Molly added that the updated rules now allow OAE use to provide more equitable screenings for students unable to respond to pure tone tests. Molly added that although the outcome fell short of the petitioner's expectations, the Board will continue to monitor screening guidelines for potential future updates.

Motion: The Board adopts the proposed rules establishing chapter 246-760 WAC as published in WSR 25-09-146, with revisions agreed upon at today's meeting, if any. The Board directs staff to file a CR-103 and establish an effective date.
Motion/Second: Member Kutz motioned/Member Flores seconded. Approved unanimously

12. RULES HEARING, REPEAL OF CHAPTER 246-366A WAC, ENVIRONMENTAL HEALTH AND SAFETY STANDARDS FOR PRIMARY AND SECONDARY SCHOOLS

Patty Hayes, Board Chair, introduced the presentation of the repeal process and the proposed changes to Chapter 246-366 WAC. Chair Hayes provided a brief background on the work of the School Rule Project (SRP) Technical Advisory Committee (TAC) and noted that the presentation includes a summary of written public comments and staff recommendations. A public hearing and Board discussion will follow the presentation.

Nina Helpling, Board staff, and Ash Noble, Board staff, introduced themselves and their role. Nina will depart on July 1 when the SRP ends, and Ash will take over the project moving forward.

Nina reviewed the history and background of the school environmental health and safety rule, the written comments received during the formal public comment period, and the next steps. Nina also discussed that the SRP TAC has met seventeen times

since August 2024 to draft the proposed Chapter 246-370 WAC. At the April 2025 Board meeting, the Board approved the SRP TAC recommendations of the proposed chapter and directed Board staff to start the repeal process for Chapter 246-366A. Board staff are also proposing to amend chapter 246-366-005 to remove a reference to Chapter 246-366A. On April 21, 2025, the CR-102 for the rules hearing was filed, and the public comments are included in the Board packets (see presentation and materials on file). Nina turned it back to Vice Chair Oshiro to start the rule hearing public comment period.

Vice Chair Oshiro announced the public hearing, directed attendees to related materials on the Board's website, read a statement with hearing instructions, and formally opened the hearing for public testimony. Each speaker was allotted three minutes. Testimony was accepted in person and via Zoom.

Michelle Larson, Board staff, called on public commenters who registered for verbal testimony via Zoom.

Laurie Layne, public commenter, supports the repeal of the rule. L. Layne said taxpayers are taxed enough, and we need to tighten our belts, and it would be nice if others would do the same. L. Layne said these projects sound wonderful, but they are unsustainable, and do them if there are no cost increases.

Michelle noted that no additional people signed up for public testimony, but 14 individuals from the public indicated their support.

Vice Chair Oshiro noted that no individuals had signed up in the room to make public comments. Vice Chair Oshiro concluded the public testimony and moved forward to the Board discussion.

Steve Kutz, Board Member, discussed the educational process during the COVID-19 pandemic. Member Kutz emphasized the importance of safe schools, making it necessary to review school environmental and safety rules. Member Kutz praised the staff and advisory committee for effectively meeting the legislative intent to protect students. Member Kutz stated that this is the core of what is needed and expressed support for their efforts, saying the team did an excellent job.

Vice Chair Oshiro thanked Chair Hayes for tireless and fearless dedication and devotion to this project that has been years in the making. Vice Chair Oshiro also thanked the SRP team, the TAC, and leadership for all their time and efforts on this project to get to the finish line.

Chair Hayes appreciated the comments and said that the timing of repealing 366A at this Board meeting is well planned and very important for several reasons. First, we know the Legislature has the proviso that has prevented the implementation of 366A. Second, it's important that the Board strongly signals to the Legislature that we understand 366A is no longer relevant. This message, along with our report, is a strong statement to send. Chair Hayes mentioned working with Executive Director Davis to brief with Senator Robinson, and it is fair to say that Senator Robinson is pleased with the current steps and shaping the next steps. Chair Hayes personally thanked the TAC

members, and said they were outstanding in truly listening and participating so the team could move forward.

Motion: The Board adopts the proposed repeal of Chapter 246-366A WAC and the amendments to chapter 246-366 WAC, as published in WSR 25-09-121 with the revisions agreed upon at today's meeting, if any, and directs staff to file a CR-103, Order of Adoption, and establish an effective date for the repeal and amendments.
Motion/Second: Member Flores/Member Kutz. Approved unanimously

Member Kutz noted that we should let former Chair Keith Greller know about this momentous action.

13. SCHOOL RULE PROJECT REPORT

Patty Hayes, Board Chair, highlighted the importance of the School Rule Project (SRP) Technical Advisory Committee (TAC) recommendations, ongoing collaboration with organizations, the Legislature, and the phased rule implementation. Chair Hayes noted the TAC included a section addressing issues like budget and the differences in school size and private schools. The Board ensured TAC members had a voice in the report. Board staff will share an overview of the draft legislative report and the Environmental Justice Assessment.

Nina Helpling, Board staff, introduced themselves and the SRP team, recognizing Chair Hayes, Project Manager Andrew Kamali, Marcus Dehart (Communications), Mary Baechle (Community Outreach), and Crystal Ogle (Administrative Coordinator). Ash Noble will take over as the policy advisor for this project after the original project team departs in July.

Ash Noble, Board staff, briefly introduced themselves and shared their background as a policy advisor to the Board.

Nina provided an overview of the proviso and its requirements for the Board, the composition of the SRP TAC, community engagement conducted, the Environmental Justice Assessment, a summary of changes, and the draft legislative report (see presentation on file). The legislative report is due on June 30, 2025.

Ash shared that they will be on a panel with Nina at the Washington State Association of Local Public Health Officials (WSALPHO) conference to discuss the SRP and its outcomes.

Nina shared that before they leave, they will have built the CR-101 packet for all three phases. Nina will also create a detailed roadmap of how the Board got here and what discussions are moving forward.

Chair Hayes said the next step is to submit the report to the Governor and Senator Robinson. We will then follow up with Senator Robinson to work on the visibility of the report either before or during the next legislative session. The Board cannot begin to implement any of these rules until the legislature releases the budget proviso. We will need communications support, including one-pagers for partners. Chair Hayes noted they will present at the upcoming Washington Association of Maintenance (WAMOA)

and WSALPHO conferences to help inform partners of this process and to answer questions or concerns.

Michelle Davis, Executive Director, said we need educational materials to explain the work and the next steps. The focus so far has been on the budget, but now we need to engage policy committees. The next steps are to contact committee chairs who discuss education policy and reconnect with the many groups engaged early in the process to share updates and offer support.

Chair Hayes emphasized the importance of engaging key TAC members and their organizations to help explain and support the phased approach. Their continued involvement is critical to moving forward, and the Board must clearly communicate how the phased implementation will work.

Ash shared that the Board's communication team is developing videos featuring TAC member testimony. Ash noted that it's important to emphasize to policymakers and the public that this will be a long process. Ash praised Nina and Andrew for their work.

Executive Director Davis said we have submitted the draft report to the Office of Financial Management. The initial feedback was supportive, but they had some questions about the state's obligation that are beyond the proviso's scope. In an ideal world, we would have a full inventory of those costs, but that is not possible within the 12 months of the proviso. Executive Director Davis shared gratitude and discussed the importance of the TAC's work, the team's efforts, and TAC members' willingness to have hard conversations.

Steve Kutz, Board Member, said the work so far has exceeded all expectations. Member Kutz praised the TAC members for coming together as a team and noted their hard discussions and compromises. Member Kutz expressed gratitude for the work completed and what is to come.

Mindy Flores, Board Member, appreciated that Nina and Andrew called each Board Member to explain the plan in detail. Member Flores asked if we have a narrative or roadmap to use for outreach to parent associations, school boards, and superintendents. Could we have that so that the key message is there for us to share?

Nina said they will work on that for Board Members, TAC members, school educators, and local health jurisdictions.

Tao Sheng Kwan-Gett, Secretary's Designee, agreed that talking points would be very helpful.

Paj Nandi, Board Member, thanked the team for their work and noted the combined TAC meeting in April was a key moment that brought everything together. Member Nandi offered to help with communications and stressed the importance of culturally and linguistically diverse materials. Member Nandi offered support for reviewing the language to ensure accessibility.

Member Kutz emphasized the need for a small team of legislators to work together and advance efforts to make schools safer for children.

Vice Chair Oshiro reflected on this past year, updates from Andrew and Nina, the extensive engagement throughout the process, and the Board team's ongoing efforts. Vice Chair Oshiro highlighted the importance of TAC members returning to their communities and the active role of parents and guardians.

Motion: The Board approves the draft report and directs staff to finalize the report in consultation with the Chair and submit it to the Office of the Governor and the appropriate committees of the Legislature.
Motion/Second: Member Kutz/Member Browning. Approved unanimously

14. BOARD MEMBER COMMENTS AND UPDATES

Steve Kutz, Board Member, thanked Executive Director Davis for bringing the team together.

Mindy Flores, Board Member, commended Board staff for their dedicated effort and contribution.

Member Kutz highlighted the dedication of Board staff.

Vice Chair Oshiro thanked everyone and adjourned the meeting.

ADJOURNMENT

Vice Chair Oshiro adjourned the meeting at 3:46 p.m.

WASHINGTON STATE BOARD OF HEALTH

_____
Patty Hayes, Chair

WASHINGTON STATE BOARD OF HEALTH

Public Comment

as of noon three business days prior to meeting

From: Bauman, Shay (SBOH)
Sent: 7/2/2025 10:08:01 AM
To: DOH WSBOH
Cc:
Subject: FW: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

attachments\D0CB17BE6C984209_SBOHLetter7.1.25.pdf

attachments\D31F7997E3324CC8_image001.png

attachments\CBA28485DC93499F_image002.png

From: Justin Stang <justinstang@PCSGA.org>
Sent: Tuesday, July 1, 2025 8:34 AM
To: Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov>; DOH WSBOH Shellfish <shellfish@sboh.wa.gov>; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov>; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov>; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov>
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org>
Subject: Re: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

External Email

Greetings,

Please find attached a letter providing our feedback on the recent survey and the proposed changes to the Washington Administrative Code. We appreciate the opportunity to share our input and welcome any follow-up discussion.

Best,

-Justin

From: Justin Stang <justinstang@PCSGA.org <mailto:justinstang@PCSGA.org> >
Sent: Friday, June 20, 2025 11:46 AM
To: Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov <mailto:Shay.Bauman@sboh.wa.gov> >; DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov <mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org <mailto:MargaretPilaro@PCSGA.org> >

Subject: Re: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

Thank you so much!

From: Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov <mailto:Shay.Bauman@sboh.wa.gov> >
Sent: Friday, June 20, 2025 11:18 AM
To: Justin Stang <justinstang@PCSGA.org <mailto:justinstang@PCSGA.org> >; DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov <mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org <mailto:MargaretPilaro@PCSGA.org> >

Subject: RE: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

Hi Justin,

Please see attached

Shay

From: Justin Stang <justinstang@PCSGA.org <mailto:justinstang@PCSGA.org> >
Sent: Thursday, June 19, 2025 12:59 PM
To: DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >; Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov <mailto:Shay.Bauman@sboh.wa.gov> >; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov <mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org <mailto:MargaretPilaro@PCSGA.org> >

Subject: Re: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

External Email

Thank you, Shay. In an effort to assist growers ahead of the deadline, is there a doc or text version of the survey that I can review? My hope is that with better understanding of the questions, I may be able to facilitate and encourage greater participation. Thanks!

-Justin□□□

From: DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >

Sent: Tuesday, June 17, 2025 10:07 AM
To: Justin Stang <justinstang@PCSGA.org <mailto:justinstang@PCSGA.org> >; DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >; Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov <mailto:Shay.Bauman@sboh.wa.gov> >; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov <mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org <mailto:MargaretPilaro@PCSGA.org> >

Subject: RE: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

Hi Justin,

Thanks for reaching out. We appreciate your feedback. The survey tool we use is used across DOH. We can share your feedback regarding the inability to save progress. We have extended the timeline to hopefully allow folks additional time to provide feedback.

The Small Business Economic Impact Statement (SBEIS) is directly informed by the feedback we receive. We look forward to reviewing responses. Thanks again.

Shay Bauman (she/her)

Policy Advisor

Washington State Board of Health

shay.bauman@sboh.wa.gov <mailto:shay.bauman@sboh.wa.gov>

(564)669-8929

Website <https://gcc02.safelinks.protection.outlook.com/?url=http%3A%2F%2Fsboh.wa.gov%2F&data=05%7C02%
|Facebook <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.facebook.com%2FWASBOH&
|Twitter <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Ftwitter.com%2FWASBOH&data=0

From: Justin Stang <justinstang@PCSGA.org <mailto:justinstang@PCSGA.org> >
Sent: Tuesday, June 17, 2025 8:13 AM
To: DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >; Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov <mailto:Shay.Bauman@sboh.wa.gov> >; Holthaus, Katitza (DOH) <katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova, Kseniya (DOH) <kseniya.efremova@doh.wa.gov <mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH) <danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Cc: Margaret Pilaro <MargaretPilaro@PCSGA.org <mailto:MargaretPilaro@PCSGA.org> >

Subject: Re: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

External Email

Dear Ash,

Thank you for the reminder and for extending the deadline for the grower survey. I truly appreciate the additional time, though I do have some concerns that the current format of the survey may limit participation and make it difficult to gather the input you're hoping for. We're currently reaching out to growers to encourage their participation in the hopes that we can assist in getting better return rates for additional perspectives.

We reviewed the survey and noted a couple of challenges. It appears the survey may take close to an hour to complete, and there does not seem to be a clear way to save progress and return later. Combined with the timing of the survey during a significant low-tide cycle, this may unintentionally create a barrier for busy growers. Moreover, are there specific materials or information that the growers should have ready and available to complete the survey?

I want to emphasize that a lack of responses should not be taken as a lack of financial impact or concern. Many growers may simply be unable to carve out the time to complete the survey as it stands. I would be glad to work with your team to consider alternative or additional ways of gathering feedback and would be happy to help convene a conversation with growers to support that effort.

Lastly, could you please clarify whether a small business economic impact report is also being developed and whether this survey is meant to inform that work, or if the two are separate?

Thanks again for your time and for your efforts to engage stakeholders in this process.

Regards,

Justin

Justin Stang (he/him)

Policy Analyst

Pacific Coast Shellfish Growers Association

120 State Ave NE #142

Olympia, WA 98501

(360) 463-0370 (mobile)

www.pcsga.org
<https://gcc02.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.pcsga.org%2F&data=05%7C

From: DOH WSBOH Shellfish <shellfish@sboh.wa.gov <mailto:shellfish@sboh.wa.gov> >

Sent: Monday, June 16, 2025 4:35 PM
To: Bauman, Shay (SBOH) <Shay.Bauman@sboh.wa.gov
<mailto:Shay.Bauman@sboh.wa.gov> >; Holthaus, Katitza (DOH)
<katitza.holthaus@doh.wa.gov <mailto:katitza.holthaus@doh.wa.gov> >; Efremova,
Kseniya (DOH) <kseniya.efremova@doh.wa.gov
<mailto:kseniya.efremova@doh.wa.gov> >; Toepelt, Danielle M (DOH)
<danielle.toepelt@doh.wa.gov <mailto:danielle.toepelt@doh.wa.gov> >
Subject: Sanitary Control of Shellfish Rulemaking Cost Survey Reminder

Good afternoon,

This is a reminder that the Board is administering a survey to better understand the costs and benefits of potential changes to chapter 246-282 WAC. Due to a low response rate thus far, we are extending the deadline for the Sanitary Control of Shellfish Rulemaking Survey. The survey will be open until Monday, June 23rd at 8:00 am.

If you have any questions or need technical assistance, please do not hesitate to reach out.

Thank you so much for your collaboration!

Ash Noble, JD, MPIA (she/her)

Policy Advisor

Washington State Board of Health

(564) 200-3685

ash.noble@sboh.wa.gov <mailto:ash.noble@sboh.wa.gov>

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Washington State Board of Health
PO Box 47990
Olympia, WA 98504

Re: Concerns Regarding Recent Survey to Shellfish Growers
Dear Members of the State Board of Health,

On behalf of the Pacific Coast Shellfish Growers Association (PCSGA), thank you for the opportunity to provide input on the recent proposed changes to WAC 246-282 and the accompanying survey issued to Washington shellfish growers. We appreciate the Department of Health's (DOH) efforts to engage with stakeholders on these important matters.

We are concerned, however, that the low survey participation should not be interpreted as an indication of a low-cost burden on the shellfish industry. Many growers found the survey difficult to complete, particularly due to the timing and the level of detail required without adequate context or clarity. These factors likely contributed to the low response rate and do not reflect a lack of concern within the industry.

The proposed changes to WAC 246-282 will result in significant costs for larger producers, especially as they relate to new tagging and documentation requirements, oyster seed size, and depletion. While smaller growers may not have indicated large direct impacts in the survey, it is important to note that nearly all rely on hatcheries and nurseries operated by larger producers for their oyster seed. Any increased costs borne by these upstream suppliers will ultimately be passed on to smaller farms, compounding the financial burden across the industry. New requirements will likely require significant changes to facilities, equipment, and growing practices. These changes come at a time when the industry is already facing tight margins and increased uncertainty.

Additionally, the new proposed rule language currently lacks critical details regarding the seed size waiver and related study. Without more information, it is difficult to fully evaluate the potential costs and implications for compliance. These uncertainties create further concern about the financial and operational feasibility of the changes.

We respectfully urge the Board not to use the survey results as a definitive measure of the rule's impact. We also request additional opportunities for meaningful engagement with stakeholders, including facilitated discussions and updated impact assessments that more accurately reflect the real and widespread consequences of the proposed changes. PCSGA further urges the Department of Health to continue outreach and collaboration with the regulated community before submitting the proposed rule for formal consideration under CR-102. Continued dialogue will help ensure that any new requirements are clearly understood, practically implementable, and aligned with the operational realities of shellfish farming in Washington.

Thank you for your attention and for your continued partnership with Washington's shellfish growers.

Sincerely,



Justin Stang
Policy Analyst, PCSGA

_____
From: bill teachingsmiles.com
Sent: 6/11/2025 8:58:05 PM
To: DOH WSBOH,Jenks, Lauren (DOH),Johnson, Laura W (DOH),Kwan-Gett, Tao (DOH),Christie, Emerson C (DOH)
Cc:
Subject: Fluoridation Safety Study



attachments\1C5E93CC5DC641A7_Drug Safety Study.pdf

External Email

Please provide this brief summary, attached pdf, regarding safety studies to the Board members and Fluoridation Panel Members.

Thank you,
Bill Osmunson DDS MPH

Bill Osmunson DDS MPH

June 11, 2025

Dear Washington State Board of Health and Fluoridation Panel

RE: Fluoridation Safety and Safety Studies

Should we judge fluoridation safety like the tobacco companies demanding absolute certainty that most people have been harmed, or from the legal and ethical side, assuring each person, dispelling any doubt, that the water is safe to drink and will be safe to drink?

RCW 43.20.050 (2)(a) answers that question and orders the Board of Health to *"Adopt rules necessary to **assure safe** . . . drinking water."*

Assuring safety means taking proactive steps to ensure secure safe water, like implementing protective measures, enforcing policies, or providing reassurances based on evidence that risks are minimized and not intentionally caused. It's forward-looking and preventative.

Proving harm, on the other hand, is about identifying, demonstrating, or providing evidence that damage, danger, or negative consequences have actually occurred. It often involves examining past events, analyzing impact, and presenting proof that fluoridation caused harm.

In other words, do we wait to see when the fluoridation lobby, those profiting from fluoride, to provide the expensive research to prove they are harming some, or do we prevent the harm by implementing protective measures before harm happens?

One seeks to prevent issues, while the other examines and establishes what has already gone wrong. The fluoridation lobby demands we add fluoride to public water until they establish that fluoridation has already gone wrong and with absolute certainty that millions have been harmed.

In contrast, the law looks forward to preventing that harm and we would be wise to obey the law.

Authorities cannot assure everyone that the fluoridated water is safe to drink. The word assure is defined as "to positively," "to confidently dispel any doubts," the water is safe and will be safe. Safe for everyone at every stage of life, regardless of how much they drink, including all other sources of fluoride, their health status, age, genetics, and other exposures of toxins, etc.

At the same time we need to determine whether we intend to protect everyone or just the statistical mean, the average person? The 1000 Genome study finds 100 or 200 fold safety factor is needed to protect everyone. For every single IQ loss, some with single-nucleotide polymorphisms (SNP) can have 10 to 30 IQ losses.[1] Safety has not happened and is not assured for the future for those with SNP variants.

I petitioned the Washington State Board of Pharmacy to designate fluoride as a poison, as provided by law, or exempt fluoride from poison laws when regulated under drug laws.[2] The Board of Pharmacy did not exempt fluoride from poison laws; however, they responded that fluoride is a legend drug requiring a doctor's prescription.[3] They further washed their hands of oversight because the legislature permits local authorities to fluoridate the water.

Just because the Legislature permitted local jurisdictions to add fluoride does not negate legal oversight. For example, the Legislature may approve a road or building but that approval does not negate the need for engineering, architects or any oversight.

Fluoridated water is an FDA-unapproved drug, dispensed without patient consent or doctor's oversight, at an unregulated dosage, without safety studies, and a contaminated industrial waste product usually from fertilizer manufacturing.

The Washington State Board of Health was told by the FDA that if approval were attempted, fluoridation would be banned. Stop for a moment. Does the FDA suggesting they would ban a product if approval were desired, assure current or future safety or dispel doubt the product is safe? No. Absolutely not.

The FDA CDER confirmed that fluoride is an unapproved drug, and their newsletter explained, *"Manufacturers of unapproved drugs are usually fully aware that their drugs*

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[1] Zhang S, Zhang X, Liu H, Qu W, Guan Z, Zeng Q, Jiang C, Gao H, Zhang C, Lei R, Xia T, Wang Z, Yang L, Chen Y, Wu X, Cui Y, Yu L, Wang A. Modifying effect of COMT gene polymorphism and a predictive role for proteomics analysis in children's intelligence in endemic fluorosis area in Tianjin, China. Toxicol Sci. 2015 Apr;144(2):238-45. doi: 10.1093/toxsci/kfu311. Epub 2015 Jan 1. PMID: 25556215.

Cui Y, Zhang B, Ma J, Wang Y, Zhao L, Hou C, Yu J, Zhao Y, Zhang Z, Nie J, Gao T, Zhou G, Liu H. Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: A school-based cross-sectional study. Ecotoxicol Environ Saf. 2018 Dec 15;165:270-277. doi: 10.1016/j.ecoenv.2018.09.018. Epub 2018 Sep 8. PMID: 30205328.

Zhao L, Yu C, Lv J, Cui Y, Wang Y, Hou C, Yu J, Guo B, Liu H, Li L. Fluoride exposure, dopamine relative gene polymorphism and intelligence: A cross-sectional study in China. Ecotoxicol Environ Saf. 2021 Feb;209:111826. doi: 10.1016/j.ecoenv.2020.111826. Epub 2020 Dec 24. PMID: 33360592.

[2] RCW 69.38.010

[3] Boyer S. MS RPh, FASHP, May 7, 2009. Executive Director, Washington State Board of Pharmacy. Letter to Dr. Osmunson.

are marketed illegally, yet they continue to circumvent the law and put consumers' health at risk."[4]

At a minimum, authorities fluoridating water must warn the public, "this drug is not FDA CDER approved," and "Do Not Swallow" and "this water is not fit for human consumption."

Give everyone the liberty to choose. If a person wants to swallow fluoride they can simply swallow a baby pea-size of fluoride toothpaste. The fluoride toothpaste label is clear, "Do Not Swallow" referring to a quarter milligram of fluoride, the same as an 11 ounce of fluoridated water. Swallowing fluoride is not safe either way, but at least a person can choose whether to swallow or not with toothpaste.

Not everyone drinks the same amount of water, and thus, dosage is not controlled. Some think 0.7 ppm is less than 1.5 ppm the NTP had concerns. True, if everyone drank the same amount of water and were adults the dosage would be less than half the NTP's concern. However, concentration is not dosage.

Safety Studies: The CDC, EPA, FDA, NSF were asked under oath whether they could provide a single safety study on fluoridation, and all said "no."

No expert or evidence has been presented to assure the Board and Department's Fluoridation Panel that there is a safety study. A study simply not reporting a risk or harm is not a safety study or proof of safety.

What is a "safety study?"

I asked that question of Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S., Scientist Emeritus and Former Director, National Institute of Environmental Health Sciences and National Toxicology Program. Her response,

"Safety is kind of like risk - requires hazard AND exposure. We don't normally talk about safety studies in environmental toxicology, while in drug studies they do.

"NTP explicitly did NOT conclude that <1.5 ppm was safe

"Personally, I am not sure if there IS a "safe" level - I am becoming more and more concerned that fluoride is like lead and there is NO safe dose, but there may be levels below which you can not measure a response.

"I don't know of any "safety" study on fluoride in drinking water. . .

"To me, observational epidemiology is where we have to look for our answers - and I think we have them. Early life exposure to fluoride is bad for the developing

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[4] FDA National Pharmacy Compliance News. July 2008. https://doh.wa.gov/sites/default/files/legacy/Documents/Pubs/347-601-NationalPharmacyComplianceNews2008.pdf?uid=63a4cada27bb2

brain. The exact water level at which this happens is not clear, but I am NOT comfortable with 0.7 ppm."

Grandjean[5] *"As a safe exposure level for fluoride in pregnancy has not been established. . . Relying on two prospective studies, maternal urine-fluoride exposure at levels commonly occurring in the general population, the joint data showed BMCL (Bench Mark Concentration Level) results about 0.2 mg/L. These results can be used to guide decisions on preventing excess fluoride exposure in vulnerable populations."*

Water fluoride concentration and urine fluoride concentration for healthy adults are ballpark similar.

The Board is required by law to assure the water is safe to drink. After more than 50 years of fluoridation in Washington State, does the Panel or the Board or the Department have in their possession, as outlined below, any:

Preclinical Safety Trials (some are available);

Clinical Safety Studies in Humans I, II, III including informed consent;

Post-Marketing Safety (Phase IV);

Regulatory requirements (FDA, EMA or ICH Guidelines); and

Safety Data Collection Tools?

Who on the Panel is a Pharmacist with drug regulatory approval experience?

Sincerely,

Bill Osmunson DDS MPH

See below for details

A **drug safety study** (also called a **pharmacovigilance** or **toxicology** study) is conducted to assess the potential risks, adverse effects, and overall safety profile of a drug before and after it is marketed. The requirements depend on the stage of drug development and regulatory guidelines, but in general, here's what's required:

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[5] Grandjean P, Hu H, Till C, Green R, Bashash M, Flora D, Tellez-Rojo MM, Song P, Lanphear B, Budtz-Jørgensen E. A Benchmark Dose Analysis for Maternal Pregnancy Urine-Fluoride and IQ in Children. medRxiv [Preprint]. 2020 Nov 4:2020.10.31.20221374. doi: 10.1101/2020.10.31.20221374. Update in: Risk Anal. 2022 Mar;42(3):439-449. doi: 10.1111/risa.13767. PMID: 33173917; PMCID: PMC7654913.

1. Preclinical Safety Studies (Before Human Trials)

These are non-clinical laboratory studies, often involving animals, and must comply with **Good Laboratory Practice (GLP)**.

- **Toxicology studies** (acute, subacute, and chronic toxicity)
- **Genotoxicity** (mutagenicity and clastogenicity)
- **Carcinogenicity studies** (for drugs intended for long-term use)
- **Reproductive and developmental toxicity**
- **Safety pharmacology** (effects on vital systems – cardiovascular, respiratory, CNS)
- **Pharmacokinetics and ADME** (absorption, distribution, metabolism, and excretion)

2. Clinical Safety Studies (In Humans)

These are done in **clinical trial phases (I–III)** and must follow **Good Clinical Practice (GCP)**.

Phase I:

- Focus: **Safety, tolerability, PK/PD** in healthy volunteers
- Required: Monitoring of **adverse events (AEs)**, **vital signs**, **laboratory tests**

Phase II & III:

- Focus: **Efficacy and continued safety monitoring**
- Required: More extensive safety data, **dose-response** relationship, **longer exposure**

Documentation:

- **Informed consent**
- **Investigator's Brochure (IB)**
- **Clinical Study Reports (CSRs)**

3. Post-Marketing Safety (Phase IV)

- Required for ongoing surveillance after drug approval
- **Spontaneous adverse event reporting**
- **Periodic Safety Update Reports (PSURs)** or **Periodic Benefit-Risk Evaluation Reports (PBRERs)**
- **Risk Management Plans (RMPs)**
- **Post-marketing safety studies (PMSS)** or **post-authorization safety studies (PASS)**

4. Regulatory Requirements

Depending on region:

- **FDA (U.S.):** IND, NDA/BLA, REMS, MedWatch, FAERS
- **EMA (EU):** EudraVigilance, RMPs, EMEA guidelines
- **ICH Guidelines:** E.g., ICH E2A–E2E (pharmacovigilance), M3 (R2) for nonclinical safety

5. Safety Data Collection Tools

- **Case report forms (CRFs)**
- **Adverse Event (AE)/Serious Adverse Event (SAE) reports**
- **Standard MedDRA Queries (SMQs)**
- **Data Safety Monitoring Boards (DSMBs)**

From: Bill Osmunson
Sent: 8/13/2025 5:05:16 PM
To: DOH WSBOH
Cc:
Subject: Re: Board Meeting Aug 20

attachments\8948688B5EBB4A8F_WSBH_Public_Comment 4_and_Regulatory_Basics.docx



attachments\850F57C5A73E412B_image001.png

External Email

Thank you Michelle,

Please add the attached to the Board's packet for public comment.

Thank you,
Bill

On Wed, Aug 13, 2025 at 9:44 AM DOH WSBOH <WSBOH@sboh.wa.gov <mailto:WSBOH@sboh.wa.gov> > wrote:

> Bill,
> Thank you, I will have you on the list!
> Here is the location information:
> Washington Department of Labor & Industries Auditorium
> 7273 Linderson Way SW in Tumwater
>
> See you next week,
>
> Michelle
>
> Michelle Larson (she/her)
>
> Communications Manager
>
> Washington State Board of Health
>
> michelle.larson@sboh.wa.gov <mailto:michelle.larson@sboh.wa.gov>
>
> c: 360-819-0047
>
> Website <https://gcc02.safelinks.protection.outlook.com/?url=http%3A%2F%2Fsboh.wa.gov%2F&data=05%7C02%
> | Facebook <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.facebook.com%2FWASBOH%

From: Bill Osmunson <billosmunson3@gmail.com <mailto:billosmunson3@gmail.com> >
Sent: Tuesday, August 12, 2025 2:43 PM
To: DOH WSBOH <WSBOH@SBOH.WA.GOV <mailto:WSBOH@SBOH.WA.GOV> >
Subject: Board Meeting Aug 20

External Email

Please put me down for public comment on the next Board meeting. I will be there in person.

Thanks,

Bill Osmunson DDS MPH

Washington State Board of Health – Public Comment and Supporting Analysis

Public Comment #4 – August 20, 2025

Bill Osmunson DDS MPH

Fluoridation Panel: Limitations and Judgment Standards Required for Fluoridation Policy

I attended the first six months of meetings as an observer to the State Board of Health's Community Water Fluoridation Panel. These observations are submitted for the public record. While I do not know the Panel's final recommendation, it is evident that the Panel's process failed in multiple areas critical to fulfilling its charge and duty.

I. Statutory Duty to Assure Safe Drinking Water

Washington law, RCW 43.20.050(2)(a), mandates that the Board of Health must "adopt rules necessary to assure safe … drinking water." This statutory language requires the Board to be able to confidently dispel reasonable doubts about water safety.

NOT A SINGLE SAFETY STUDY WAS REVIEWED BY THE PANEL

NOT A SINGLE HIGH QUALITY RANDOMIZED CONTROLLED TRIAL OF EFFICACY WAS REVIEWED BY THE PANEL. Efficacy is unknown, uncertain, incomplete without quality research. No one on the panel was competently trained to evaluate the research on efficacy.

In clinical practice, malpractice is determined when a provider fails to meet the standard of more likely than not (≥51% confidence) that their actions were safe and appropriate—not the 99%+ proof-of-harm threshold that some Panel members appeared to require.

The Board's legal duty is safety assurance, not benefit–risk balancing. The statute does not authorize adding a drug to public water without first proving safety under all intended conditions of use.

Discussion was made around 1.5 ppm fluoride in water is the safety threshold. What margin of safety was used? None. The NTP did not suggest below 1.5 ppm was safe or there was any safe dosage. The 1.5 ppm was a "statistical mean" and made no protection for those drinking more water or infants or chemically sensitive or genetic sensitivities.

THE BOARD CANNOT HONESTLY, SCIENTICALLY OR LEGALLY ASSURE THE PUBLIC THE WATER IS SAFE TO DRINK.

II. The Panel's Charge and Its Shortcomings

The Board's charge to the Panel stated:

"For those of us in the governmental public health system to listen, learn, and develop our own way to consider all relevant science in our discussions of community water fluoridation policy and recommendations. Consolidate what we learn and interpret the science into a briefing so that the State Board of Health can consider it in potential policy action."

"Develop our own way" –The FDA, EPA, Washington State Board of Pharmacy, the U.S. Pharmacopeia, and the FD&C Act (21 U.S.C. § 321(g)(1)) all recognize fluoride as a drug when its intended use is to prevent dental caries.

Lack of Regulatory Expertise – Panel members were dedicated professionals but none were drug regulatory experts, trained in FDA drug approval requirements, or equipped with the procedures necessary to authorize a drug for population-wide ingestion. This inherently limits the Board's ability to fulfill its statutory duty to assure safe drinking water.

Avoiding Established Authority – The FDA's Center for Drug Evaluation and Research (CDER) has decades of experience evaluating safety and efficacy. Developing a "new way" in Washington would take years and substantial resources. Alternatively, the Board could simply defer to FDA expertise. Indeed, over a decade ago, when consulted, FDA stated it would ban fluoridation as an unapproved drug—a position wholly inconsistent with assuring the public that the water is safe.

If Going Alone, Higher Standards Apply – If the Board insists on an independent review, it must adopt stricter safety standards than those of FDA, not weaker. This is especially true for involuntary, population-wide drug exposure.

III. "Consider All Relevant Science" – Undefined Judgment Standard
The instruction to "consider all relevant science" is too vague without defining what standard of judgment applies:

Will science be judged as drug safety reviewers (FDA) do, requiring affirmative proof of safety?

Or as contaminant regulators (EPA) do, acting only with near-certainty of severe harm?

Without clear instructions, Panel members defaulted to inconsistent standards—some demanding absolute certainty of harm before concern, others applying the precautionary approach. This fractured approach undermines any claim of consensus.

IV. Critical Omissions in the Panel's Work
No High-Quality Safety Studies Reviewed – The Panel did not review a single high-quality safety study on fluoride's impact on the developing brain or on any organ system. Evidence was provided in recorded sworn testimony deposition that neither the FDA CDC EPA nor NSF had a single safety study on fluoride ingestion.

This is inconsistent with RCW 43.20.050's mandate to assure safety. The Panel reviewed studies reporting harm, but no quality safety study. In fact, I doubt many even understand the difference. Absence of proof of harm is not proof of safety. The National Research Council (2006) report explicitly called for new safety research on neurotoxicity, endocrine effects, and sensitive populations. These have not been done.

No High-Quality Efficacy Trials – No randomized controlled trials (RCTs) on CWF's caries-preventive effect were reviewed, despite the Cochrane Collaboration (2015; 2024 update) concluding that the quality of evidence for CWF's benefit is low.

No Consideration of Vulnerable Populations – The Panel did not evaluate safety for infants, pregnant women, people with kidney disease, or genetic subgroups with increased fluoride sensitivity.

No Safe Dose Determination – The Panel did not determine a safe dose or address the wide range of total fluoride exposures from all sources. Instead, it focused narrowly on average intake without applying any safety factor or margin of error, contrary to standard toxicological practice.

Inadequate Timeframe – The NRC's 2006 fluoride review took three years, a substantial budget, and expert panels. The Washington Panel's six-month process was insufficient to review "all relevant science."

Public Comment Limitations – I was not permitted to present my full analysis to the Panel, and it is unclear if all members received or reviewed the material submitted. Nor was I able to provide evidence to refute some poor quality research.

V. Conclusion on the Panel

The Board cannot fulfill its statutory duty to "assure safe drinking water" by relying on a Panel that:

Lacked drug regulatory expertise,
Did not review high-quality safety or efficacy evidence,
Applied inconsistent judgment standards, and
Operated under inadequate time and scope to consider all relevant science.

Given the FDA's own acknowledgment that fluoridation would not meet drug approval requirements, the only defensible course of action—scientifically, ethically, and legally—is for the Board to withdraw its recommendation for community water fluoridation until FDA-approved safety and efficacy data exist.

CHARGE TO THE PANEL SHOULD HAVE INCLUDED THE FOLLOWING GUIDANCE

A. Statutory Definition of a Drug

Federal Food, Drug, and Cosmetic Act (FD&C Act), 21 U.S.C. § 321(g)(1):

(A) articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them;
(B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals;
(C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and
(D) articles intended for use as a component of any article specified in clause (A), (B), or (C).

Application to Fluoride in Drinking Water:
When fluoride is added to public drinking water with the stated intent to prevent dental caries, it meets the definition in § 321(g)(1)(B). By statute, this places the substance under FDA jurisdiction as a drug, requiring New Drug Application (NDA) approval before marketing or distribution for human consumption.

B. Comparison of FDA and EPA Safety Judgment Standards

Criteria	FDA Drug/Additive Standard	EPA Contaminant Standard
Regulatory Mandate	Requires reasonable assurance of safety before market approval or use.	Requires prevention of unreasonable risk from contaminants; action when certainty of severe harm.
Burden of Proof	On the manufacturer/proponent to demonstrate safety before exposure.	On the regulator to demonstrate significant harm before restricting/removing.
Evidence Required	Positive, reliable evidence showing no unacceptable harm.	Evidence of harm in humans or compelling epidemiological data.
Treatment of Uncertainty	Precautionary: resolve in favor of protection; absence of harm is not proof of safety. Safety Factor.	Reactive: often resolved in favor of continued use until harm proven.

Scope of Effects	All adverse effects including subtle, chronic, developmental, reproductive, neurotoxic.	Primarily serious, overt, well-established harms (e.g., severe skeletal fluorosis).
Timing of Action	Pre-market — only after safety is demonstrated.	Post-market — after harm detected or significant risk confirmed.
Application to Fluoride	Would require NDA-level safety data before public water addition.	Allows addition to achieve ~0.7 mg/L without pre-market safety proof.

C. Key Points in Practice

- **Intent of use** is central.
    - If a substance is marketed or used **with the intent** to diagnose, treat, mitigate, cure, or **prevent** disease — it is a **drug** under the FD&C Act.
- **Includes preventive use** — so if fluoride is added to drinking water **with the stated intent of preventing dental caries**, or well known to the public with intent to prevent dental caries, it falls under **21 U.S.C. § 321(g)(1)(B)**.
- **Not dependent on form** — drugs can be tablets, injections, solutions, or even **diluted in drinking water**.
- **Components of drugs** are also regulated as drugs — meaning even a raw chemical intended for drug use is a "drug" legally.

D. First RTCs to determine efficacy at a specific dosage and label. Only after high confidence of efficacy, then the CMC requirements must be met.

FDA CMC (Chemical, Manufacturing, Control) Requirements vs. Current Fluoridation Practice

FDA NDA CMC Requirement	What This Means in Drug Regulation	Current Practice in Community Water Fluoridation
1. Drug Substance Identification	Chemical name, structure, molecular formula, CAS number, physical/chemical properties, impurity profile.	Usually only bulk chemical name is known (e.g., hydrofluorosilicic acid), often without full impurity profile or detailed manufacturing origin.
2. Drug Substance Manufacture	FDA-registered manufacturer, GMP-compliant facility, full manufacturing process and quality control data.	Industrial suppliers not registered as drug manufacturers; source often from phosphate fertilizer byproduct streams. No GMP oversight by FDA.
3. Specifications & Testing	Tight limits on active ingredient content, impurities, and contaminants; validated analytical methods.	Limited specifications (AWWA/NSF standards), not FDA-validated. Testing may occur only at point-of-entry, not in distributed water at consumer tap.
4. Drug Product Composition	Complete formulation, including all active/inactive ingredients and concentrations.	"Formulation" is bulk water plus added fluoride compound; concentration target is ~0.7 mg/L, but other substances (arsenic, lead, radionuclides) may be present without full disclosure.
5. Dosage Form & Strength	Clearly defined per unit dose (e.g., mg per tablet); dosing accuracy verified in each batch.	No per-person dosing control; individual intake varies widely by age, health status, and water consumption.
6. Manufacturing Process & Batch Records	Detailed, validated process; batch records kept for each lot; FDA inspection possible.	No validated process under FDA authority; water utility records are operational logs, not NDA-quality batch documentation.
7. In-Process Controls	Sampling and testing during manufacturing to ensure consistency before final	Fluoride concentration may be checked periodically (e.g., daily grab samples), but frequency and method

FDA NDA CMC Requirement	What This Means in Drug Regulation	Current Practice in Community Water Fluoridation
	release.	vary; no uniform federal release criteria.
8. Facility Registration & GMP Compliance	All facilities registered with FDA; subject to cGMP inspections and enforcement.	Municipal water plants are not FDA-registered or inspected as drug manufacturing facilities.
9. Container/Closure System	Data proving compatibility (no leaching/reactivity), stability during storage.	Bulk fluoride shipped in tanks/drums; no FDA stability data for interaction with pipes or storage tanks.
10. Stability Data	Shelf-life studies under ICH conditions; degradation products identified and assessed.	No long-term stability studies for fluoride in distribution systems; concentration can change between plant and tap.
11. Analytical Method Validation	Methods for potency, purity, identity validated per ICH/FDA guidance.	Field test kits may not meet FDA validation standards; lab methods vary by jurisdiction.
12. Microbiological Quality	Microbial limits or sterility data for certain dosage forms.	Not evaluated for fluoride additive; microbial safety assessed separately for water as a whole under EPA rules.
13. Labeling	Drug label with dosage, indications, contraindications, warnings, side effects.	No labeling provided to consumers; exposure is involuntary and unlabeled.
14. Storage & Handling Conditions	Defined temperature, light, and humidity requirements to maintain product quality.	Bulk fluoride often stored in industrial tanks; no FDA-verified stability or storage condition data.
15. Post-Approval Changes & Reporting	NDA supplements filed for any change in manufacture, composition, or labeling.	Fluoride source or process changes not reported to FDA; changes may occur without public disclosure.

- ---
- **Summary Observation:**
  If community water fluoridation were treated as an **FDA-regulated oral drug**, it would **fail to meet nearly every CMC requirement**. No NDA exists, no FDA-registered facility manufactures the "final drug product" (fluoridated drinking

water), and no batch-level dosing control exists for individuals. This creates a **regulatory gap** where a substance intended for disease prevention is **administered without the legal safeguards required for all other drugs**.

D. Conclusion

Under federal law, fluoride added to drinking water with intent to prevent disease meets the statutory definition of a drug. FDA standards require pre-market proof of safety and full CMC compliance for such substances. The current regulatory approach instead applies EPA's harm-based contaminant standard, leaving a significant legal and public health gap. Correcting this gap is essential to align with federal drug law, ensure ethical public health practice, and protect vulnerable populations.

Our Public Health Profession and Dental Profession credibility are at stake. If the Board is unwilling to obey the law assuring safe drinking water and FDA CDER approval, then the public has no reason to believe advice regarding other public health policies.

Sincerely,

Bill Osmunson DDS MPH

From: Gerald Braude
Sent: 8/15/2025 10:29:45 AM
To: DOH WSBOH
Cc:
Subject: Gerald Braude public comment



attachments\F36BE9590D9D4810_35dd3198.png

External Email

Dear Michelle:

Please sign me up for in-person public comment for this Wednesday, August 20 Board of Health meeting.

Below are a few written comments as well.

Thank you,

Gerald Braude

Port Townsend

The fact is both of the two deaths that Tao Kwan-Gett mentioned at the April 9 Board of Health meeting were not from measles but were due to medical error, the third leading cause of death in the United States, an alarming topic that the Board of Heath has never discussed.

Medical error—the third leading cause of death in the US | The BMJ
<https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.bmj.com%2Fcontent%2F353

Only one death from measles has occurred over the past decade, but, during this same period, VAERS shows seventy-three deaths
<https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fsubstack.com%2Fredirect%2F16c
7163-4591-99d6-
3fe96c954045%3Fj%3DeyJ1IjoiMTByMnA3In0.RALPmDxM8D0L5FfNiR0WshM8To-
iQhoO9mvADKCCSWQ&data=05%7C02%7Cwsboh%40sboh.wa.gov%7Cd3a349dcda9e4ed1df4d08dddc215
following the measles vaccines.

Four of those deaths
<https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fsubstack.com%2Fredirect%2F16c
7163-4591-99d6-
3fe96c954045%3Fj%3DeyJ1IjoiMTByMnA3In0.RALPmDxM8D0L5FfNiR0WshM8To-
iQhoO9mvADKCCSWQ&data=05%7C02%7Cwsboh%40sboh.wa.gov%7Cd3a349dcda9e4ed1df4d08dddc215
have occurred here in Washington.

The most recent death in Washington following the measles vaccine occurred on February 22, 2020. Here is the beginning portion of the submitted write-up:

Patient is a previously healthy 13 month old boy who presented with respiratory failure, then developed ARDS and multiorgan dysfunction on VA ECMO, requiring vasoactive support and CRRT. Subsequently found to have multiple disseminated viral infections, including HSV, adenovirus, and low level positive CMV and EBV. Suspected immunodeficiency, workup pending. In setting of recent MMR and varicella vaccinations, critical illness, and suspected immunodeficiency, workup for disseminated vaccine strain measles sent at CDC. Positive for vaccine-strain measles from nasopharynx and urine.

--

a

Search Results

From the 4/25/2025 release of VAERS data:

Found 73 cases where Vaccine targets Measles (MEA or MER or MM or MMR or MMRV) and Patient Died and Submission Date from '2014-01-01' to '2024-12-31'

Government Disclaimer on use of this data

Table

Age	Count	Percent
< 6 Months	3	4.11%
6-11 Months	2	2.74%
1-2 Years	38	52.05%
3-5 Years	7	9.59%
6-17 Years	5	6.85%
Unknown	18	24.66%
TOTAL	73	100%

Case Details

_____

From: tweetfamily@comcast.net
Sent: 8/15/2025 11:55:20 AM
To: DOH WSBOH
Cc:
Subject: Aug 20, 2025 WSBOH Public Comment, no proof of safety of fluoridation chemicals

attachments\1F76DC26FB1F4A82_Aug 20,2025 WSBOHpubliccomment.pdf

attachments\FF579A85240F48E5_Safety Data Sheets.pdf

External Email

Attached is a "Safety" data sheet from 2020 from the city of Battleground, WA the only one found in public records. No proof of safety, many concerns raised.
See also my comments about potential for harm to vulnerable members, babies, children, pregnant women, those hypersensitive to fluoridation chemicals.
Submitted by Margaret Tweet, WA resident

On September 24, 2024 a US federal court deemed fluoridation of Public Tap Water an "unreasonable risk" to the health of children, and the EPA must regulate it as such. decision See ruling excerpt:

"The issue before this Court is whether the Plaintiffs have established by a preponderance of the evidence that the fluoridation of drinking water at levels typical in the United States poses an unreasonable risk of injury to health of the public within the meaning of Amended TSCA. For the reasons set forth below, the Court so finds. Specifically, the Court finds that fluoridation of water at 0.7 milligrams per liter ("mg/L") – the level presently considered "optimal" in the United States – poses an unreasonable risk of reduced IQ in children.. the Court finds there is an unreasonable risk of such injury, a risk sufficient to require the EPA to engage with a regulatory response…One thing the EPA cannot do, however, in the face of this Court's finding, is to ignore that risk."

During the trial, top scientific experts who advised the EPA on understanding and setting hazard levels for other major environmental toxins and who conducted gold-standard "cohort" studies on the link between fluoride and low IQ in children testified for FAN.

They explained the NTP's findings and presented evidence from their own research showing neurotoxic risks - particularly to pregnant women, formula-fed infants and children- posed by water fluoridation.

Fluoride in Water Poses 'Unreasonable Risk' to Children, Federal Judge Rules

Meta-analysis

The meta-analysis is an in-depth statistical analysis of 74 epidemiological studies specifically on children's IQ and fluoride exposure from the recently published NTP Monograph: a Systematic Review of Fluoride Exposure and Neurodevelopment and Cognition, conducted by the same scientists.

Of the 74 studies, 22 were rated as high quality (low risk of bias) and 52 were rated as low quality (high risk of bias). Studies were conducted in 10 countries, including Canada, China, Denmark, India, Iran, Mexico, Pakistan, New Zealand, Spain, and Taiwan. None of the studies were conducted in the United States. These studies measured fluoride in drinking water or in urine. The measurement of fluoride in urine is an estimate of total fluoride exposure.

The meta-analysis found **a statistically significant association between higher fluoride exposure and lower children's IQ scores**, showing that the more fluoride a child is exposed to, the more likely that child's IQ will be lower than if they were not exposed.

The meta-analysis found that **for every 1 mg/L increase in urinary fluoride, there is a decrease of 1.63 IQ points in children.**

Using data from the high-quality studies, the analyses found that fluoride exposure was associated with lower IQ scores at levels below 1.5 mg/L fluoride measured in urine or drinking water. However, there were few studies, and therefore uncertainty, in the relationship below 1.5 mg/L when fluoride was measured only in drinking water. There were not enough data to determine if 0.7 mg/L of fluoride exposure in drinking water affected children's IQ. **https://ntp.niehs.nih.gov/research/assessments/noncancer/completed/fluoride**

*Do **Not impose fluoridation chemicals** upon residents who object to or cannot tolerate fluoridation chemicals. What other chemicals are included in the bags of sodium fluoride? A single batch test from the city of Battle Ground was found, from 2020 that is attached and is concerning.

A new perspective on metals and other contaminants in fluoridation chemicals*

PMCID: PMC4090869 PMID: 24999851
https://pmc.ncbi.nlm.nih.gov/articles/PMC4090869/

Abstract

Background:

Fluoride additives contain metal contaminants that must be diluted to meet drinking water regulations. However, each raw additive batch supplied to water facilities does not come labeled with concentrations per contaminant. This omission distorts exposure profiles and the risks associated with accidents and routine use.

Objectives:

This study provides an independent determination of the metal content of raw fluoride products.

Methods:

Metal concentrations were analyzed in three hydrofluorosilicic acid (HFS) and four sodium fluoride (NaF) samples using inductively coupled plasma-atomic emission spectrometry. Arsenic levels were confirmed using graphite furnace atomic absorption analysis.

Results:

Results show that metal content varies with batch, and all HFS samples contained arsenic (4.9–56.0 ppm) or arsenic in addition to lead (10.3 ppm). Two NaF samples contained barium (13.3–18.0 ppm) instead. All HFS (212–415 ppm) and NaF (3312–3630 ppm) additives contained a surprising amount of aluminum.

Conclusions:

Such contaminant content creates a regulatory blind spot that jeopardizes any safe use of fluoride additives.

Mullenix PJ. A new perspective on metals and other contaminants in fluoridation chemicals. Int J Occup Environ Health. 2014 Apr-Jun;20(2):157-66. doi: 10.1179/2049396714Y.0000000062. Epub 2014 Mar 20. PMID: 24999851; PMCID: PMC4090869.

Florida Becomes Second State to Ban Fluoride in Public Drinking Water

Ashley Malin, Ph.D., assistant professor in the University of Florida's Department of Epidemiology, also spoke at the press conference. She is the lead author of a recent **study of women and children** in Los Angeles that found children born to women exposed during pregnancy to fluoridated drinking water were more likely to have neurobehavioural problems.

Malin said there have been "major milestones" in research on fluoride's effects on neurodevelopment in the last decade, and particularly in the last year. She added:

"With more and more studies finding associations of **fluoride exposure** with adverse neurological outcomes, even at levels that we commonly encounter in Florida, the findings of these studies point to an imminent need for policy change and the policy change happening in Miami-Dade today with the ultimate signing of this bill, is one that will protect the brains of children in Florida from unreasonable risk of harm for generations to come."

When Ladapo recommended that Florida communities end fluoridation last year, he said a **landmark ruling** in September 2024 by a California federal judge prompted him to review the science.

In that ruling, U.S. District Judge Edward Chen concluded water fluoridation at current U.S. levels poses an "**unreasonable risk**" to children's health. Chen ordered the U.S. Environmental Protection Agency to take regulatory action in light of recent scientific findings.

The ruling stemmed from a **lawsuit** filed in 2017 by **Fluoride Action Network**, **Moms Against Fluoridation**, **Food & Water Watch** and individual parents and children.

It also followed the publication in August 2024 of a key report by the U.S. Department of Health and Human Services' **National Toxicology Program**. The report concluded that higher levels of fluoride exposure in drinking water are consistently **linked to lower IQ in kids**.

In October 2024, an updated **Cochrane Review** concluded that adding fluoride to drinking water provides very limited, if any, **dental benefits**, especially compared with 50 years ago.

See: https://childrenshealthdefense.org/defender/florida-second-state-ban-fluoride-public-drinking-water/

Children Whose Mothers Were Exposed During Pregnancy to Fluoridated Tap Water at Higher Risk of Neurobehavioral Problems

Children born to women exposed during pregnancy to fluoridated drinking water were more likely to have neurobehavioural problems, according to a study published today in JAMA Network Open.

In the first U.S.-based cohort study to examine this link, researchers from the Keck School of Medicine at the University of Southern California (USC), the University of Florida and Indiana University followed 229 mother-child pairs from pregnancy until the children were 3 years old.

They found that a 0.68 milligram per liter (mg/L) increase in fluoride exposure during pregnancy was associated with nearly double the chance of a child, by age 3, exhibiting neurobehavioral problems at or near a level that meets the criteria for a clinical diagnosis.

Dr. Howard Hu, Flora L. Thornton Chair and Professor of Population and Public Health Sciences at USC and co-author of the study told The Defender the study's findings are concerning because the women were not exposed to particularly high fluoride levels.

Their fluoride exposure came primarily from fluoridated tap water in Los Angeles, which has water fluoridation at 0.7 mg/L — typical for fluoridation levels in most U.S. cities and towns and approximately the same level found to have effects in the study.

"When you add this to all the other studies that have been done on this subject in the last few years," Hu said, "it creates a body of evidence, which — in conjunction with the basic science looking at how fluoride may be toxicologically active on the brain — suggests that the impact of fluoride on neurobehavioral development problems is causal. It's not just an epidemiological association."

The authors concluded that it may be necessary to "establish recommendations for limiting exposure to fluoride from all sources during the prenatal period, a time when the developing brain is known to be especially vulnerable to injury from environmental insults."

See https://childrenshealthdefense.org/defender/pregnant-mothers-fluoridated-tap-water-children-higher-risk-neurobehavioral-problems/

In May 2016, the Washington State Department of Health changed the fluoridation requirement to a target of 0.7 mg/L from the previous target of 0.7-1.2 mg/L. (Fluoride is NOT required. Washougal and other cities in WA state do NOT add fluoride to the water.)

2024 Camas Water Quality Report Fluoride, a contaminant, detected at .12-1.24 mg/Liter, above the goal range.

https://www.cityofcamas.us/publicworks/page/water-quality-reports

Maternal Urinary Fluoride Levels of a Large Pregnancy Cohort in the United States: Findings from the ADORE Study

Discussion: MUFsg in this population of midwestern US women exceeds the safety benchmark for pregnancy. While we cannot account for all sources of fluoride, MUFsg was correlated to PWS fluoridation. Because so many exceeded the safety benchmark for MUFsg, there is a need for MUFsg evaluation in other US regions, especially where the PWS fluoridation exceeds US Department of Health and Human Services recommendations (≥0.7mg/L). https://doi.org/10.1289/EHP14711.

https://pubmed.ncbi.nlm.nih.gov/40043234/

Infants Exposure To Fluoride

The most vulnerable to fluoride's toxicity is a developing fetus or an infant due to their small size. Infants receive up to 400% more fluoride (per pound of body weight) than adults consuming the same level of fluoride in water. Not only do infants receive a larger dose, but they also have an impaired ability to excrete fluoride through their kidneys. Healthy adults can excrete more than 50% of an ingested fluoride dose; infants, by contrast, can only excrete 15 to 20%. This leads to a greater build-up of fluoride in the body, and may help explain why infants fed formula made with fluoridated water suffer higher rates of dental fluorosis, a discoloration of the teeth caused by excessive fluoride ingestion during childhood.

Teeth are not the only tissue that can be affected by fluoride exposure during infancy. A baby's blood brain barrier is not fully developed at birth, and this allows fluoride, a neurotoxin, greater access to the brain than in later periods in life.

Fluoride Hypersensitivity Hypersensitive reactions have been reported for both topical fluorides (e.g., toothpaste) and systemic fluorides (e.g., fluoride supplements and fluoridated water).

See Health Conditions that may worsen with fluoride exposure, eg kidney, thyroid, diabetes…

https://fluoridealert.org/key-topics/health-effects-of-fluoride/

Fluorosis Rates Are on the Rise

Before the widespread use of fluoride in dentistry, dental fluorosis was rarely found in western countries. Today, with virtually every toothpaste now containing fluoride, and most U.S. water supplies containing fluoride chemicals, dental fluorosis rates have reached unprecedented levels. The CDC's National Health and Nutrition Examination Survey (NHANES) has consistently found skyrocketing rates of dental fluorosis. In 2004, the agency reported that 41% of adolescents (12 to 15) had dental fluorosis, then in the CDC's 2012 survey they found that the rate jumped significantly to 65+% of adolescents with dental fluorosis. Now, according to a recent study (Yang, June 2021) published in the journal Ecotoxicology and Environmental Safety using the data from the NHANES 2015-16 survey, the "prevalence of dental fluorosis [has increased to] 70% in U.S. children." '

See https://fluoridealert.org/?article=dental

Health Risks outweigh benefits. Topical fluoridation, toothpaste, etc is available. Halt water fluoridation to:

* **Reduce the risk of health harm,** to vulnerable residents especially, babies, children, pregnant women, and those with hypersensitivity to fluoride or health conditions related to kidney, thyroid and other issues.

* **expensive filter systems to remove fluoridation chemicals** that the city adds to the water are a burden

*Few notification requirements for known contaminants such as sodium fluoride exist. An annual water quality report includes a range of levels reported.

Please, do NOT add sodium fluoride contaminants to tap water. The potential for adverse health impacts on vulnerable populations, babies, children, those who are hypersensitive to the products added, and those with various health conditions is high.

Other means to apply fluoride products topically to teeth are readily available and affordable.

SAFETY DATA SHEETS

According to American OSHA Hazard Communication Standard 2012 (29 CFR 1910.1200)

Version: 1.0
Creation Date: Dec. 22, 2020
Revision Date: Dec. 30, 2020

1. Identification

1.1 Product identifier

Product name Sodium Fluoride

1.2 Other means of identification

Product number -
Other names Sodium fluoride

1.3 Recommended use of the chemical and restrictions on use

Identified uses It is used to fluoridate water. Also be used to enhance the strength of teeth by the formation of fluorapatite. Also can be used as disinfectant, antiseptic, insecticide, also used in enamel, wood preservation, medicine, metallurgy and fluoride production, etc.
Restrictions Industrial use only.

1.4 Details of the supplier of the safety data sheet

Distributed by:
Cascade Columbia Distribution Company
6900 Fox Avenue South, Seattle WA 98108
Phone: +1 (206) 282-6334
Emergency phone: CHEMTREC 1-800-424-9300 (USA)
CANUTEC: 1-613-996-6666 (CANADA)

2. Hazard(s) identification

2.1 GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Acute toxicity - Oral, Category 3
Skin irritation, Category 2
Eye irritation, Category 2

2.2 GHS label elements, including precautionary statements

Pictogram(s)



Signal word Danger
Hazard statement(s) H301 Toxic if swallowed
H315 Causes skin irritation
H319 Causes serious eye irritation

Precautionary statement(s)
Prevention P264 Wash skin thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
Response P301+P310 IF SWALLOWED: Immediately call a POISON CENTER/ or Doctor.
P330 Rinse mouth.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P332+P313 If skin irritation occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before reuse.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 If eye irritation persists: Get medical advice/attention.
Storage P405 Store locked up.
Disposal P501 Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS

3. Composition/information on ingredients

3.1 Substances

Chemical name	Common names and synonyms	CAS number	EC number	Concentration
Sodium fluoride	Sodium Fluoride	7681-49-4	231-667-8	>98%

4. First-aid measures

4.1 Description of necessary first-aid measures

If inhaled

Fresh air, rest. Half-upright position. Refer for medical attention.

Following skin contact

Remove contaminated clothes. Rinse skin with plenty of water or shower. Refer for medical attention .

Following eye contact

First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then refer for medical attention.

Following ingestion

Rinse mouth. Induce vomiting (ONLY IN CONSCIOUS PERSONS!). Give one or two glasses of water to drink. Refer for medical attention .

4.2 Most important symptoms/effects, acute and delayed

no data available

4.3 Indication of immediate medical attention and special treatment needed, if necessary

no data available

5. Fire-fighting measures

5.1 Suitable extinguishing media

Use dry chemical, carbon dioxide or alcohol-resistant foam.

5.2 Specific hazards arising from the chemical

Not combustible. Gives off irritating or toxic fumes (or gases) in a fire.

5.3 Special protective equipment and precautions for fire-fighters

In case of fire in the surroundings, use appropriate extinguishing media. In case of fire: keep drums, etc., cool by spraying with water.

6. Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Personal protection: particulate filter respirator adapted to the airborne concentration of the substance. Sweep spilled substance into covered containers. Carefully collect remainder. Then store and dispose of according to local regulations.

6.2 Environmental precautions

Prevent further spillage or leakage if it is safe to do so. Do not let the chemical enter drains. Discharge into the environment must be avoided.

6.3 Methods and materials for containment and cleaning up

Collect and arrange disposal. Keep the chemical in suitable and closed containers for disposal. Remove all sources of ignition. Use spark-proof tools and explosion-proof equipment. Adhered or collected material should be promptly disposed of, in accordance with appropriate laws and regulations.

7. Handling and storage

7.1 Precautions for safe handling

NO open flames. NO contact with hot surfaces.

7.2 Conditions for safe storage, including any incompatibilities

Separated from acids and food and feedstuffs.

8. Exposure controls/personal protection

8.1 Control parameters

Occupational Exposure limit values

TLV: 2.5 mg/m3, as TWA; A4 (not classifiable as a human carcinogen); BEI issued.EU-OEL: (as F): 2,5 mg/m3 as TWA.MAK: (inhalable fraction, as F): 1 mg/m3; peak limitation category: II(4); skin absorption (H); pregnancy risk group: C

Biological limit values

no data available

8.2 Appropriate engineering controls

Ensure adequate ventilation. Handle in accordance with good industrial hygiene and safety practice. Set up emergency exits and the risk-elimination area.

8.3 Individual protection measures, such as personal protective equipment (PPE)

Eye/face protection

Wear face shield or eye protection in combination with breathing protection if powder.

Skin protection

Protective gloves.

Body Protection

Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection

Use ventilation (not if powder), local exhaust or breathing protection.

9. Physical and chemical properties

Appearance	White powder
Odor	no data available
Odor threshold	no data available
pH	no data available
Melting point/freezing point	993°C
Initial boiling point and boiling range	1700°C
Flash point	Not°Considered to be a fire hazard
Evaporation rate	no data available
Flammability	no data available
Upper/lower flammability or explosive limits	no data available
Vapor pressure	1.4 mm Hg (0 °C)
Vapor density	no data available
Relative density	2.8 g/cm³
Solubility(ies)	Solubility in water, g/100ml at 20°C: 4.0
Partition coefficient n-octanol/water	no data available
Auto-ignition temperature	no data available
Decomposition temperature	no data available
Viscosity	no data available

10. Stability and reactivity

10.1 Reactivity

no data available

10.2 Chemical stability

no data available

10.3 Possibility of hazardous reactions

Decomposes on contact with hot surfaces or flames. This produces toxic and corrosive fumes. Reacts with acids. This produces toxic and corrosive fumes.

10.4 Conditions to avoid

no data available

10.5 Incompatible materials

no data available

10.6 Hazardous decomposition products

no data available

11. Toxicological information

Acute toxicity

- LD50 Oral - rat - 31 mg/kg
- TDLo Oral - Human - 0.214mg/kg
- Remarks: Behavioral: Headache. Gastrointestinal: Changes in structure or function of salivary glands.
- TDLo Oral- Human- female - 7 mg/kg
- Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste): Eye: Ptosis, Cyanosis
- Inhalation: no data available.
- Dermal: no data available.
- LD50 Intravenous - rat- 26 mg/kg
- Remarks: Nutritional and Gross Metabolic: weight loss and decreased weight again.

Skin corrosion/irritation

Irritating to skin

Serious eye damage/irritation

Eyes-rabbit
Result: Eye irritation - 24 h
Remarks: Moderate eye irritation

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

This product is or contains a component that is not classifiable as to its carcinogenicity based on its IARC, ACGHI,NTP,or EPA classification.

IARC: 3-Group 3:Not classifiaable as to its carcinogenicity to humans (sodium fluoride).

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA:No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carginogen by OSHA.

Reproductive toxicity

no data available

STOT-single exposure

The substance is irritating to the eyes, skin and respiratory tract. Ingestion could cause hypocalcaemia and hypokalaemia. This may result in central nervous system disorders and cardiac disorders.

STOT-repeated exposure

The substance may have effects on the bones and teeth. This may result in fluorosis.

Aspiration hazard

Evaporation at 20°C is negligible; a harmful concentration of airborne particles can, however, be reached quickly on spraying or when dispersed, especially if powdered.

Additional Information
Prolonged or repeated exposure can cause,
Damage to the lungs.
Liver- Irregulartities -Based on Human Evidence
Liver- Irregulartities -Based on Human Evidence

12. Ecological information

12.1 Toxicity

- Toxicity to fish: LC50 - Oncorhynchus mykiss (previous name: Salmo gairdneri) - 107.5 ppm - 96 h.
- Toxicity to daphnia and other aquatic invertebrates: EC50 - trichoptera aquatic larvae - 26 - 48 mg/L - 96 h.
- Toxicity to algae: EC50 - various algae species - 43 mg/L - 96 h.
- Toxicity to microorganisms: NOEC - various - 83 mg/L - 48 h.

12.2 Persistence and degradability

no data available

12.3 Bioaccumulative potential

no data available

12.4 Mobility in soil

no data available

12.5 Other adverse effects

no data available

13. Disposal considerations

13.1 Disposal methods

Product

The material can be disposed of by removal to a licensed chemical destruction plant or by controlled incineration with flue gas scrubbing. Do not contaminate water, foodstuffs, feed or seed by storage or disposal. Do not discharge to sewer systems.

Contaminated packaging

Containers can be triply rinsed (or equivalent) and offered for recycling or reconditioning. Alternatively, the packaging can be punctured to make it unusable for other purposes and then be disposed of in a sanitary landfill. Controlled incineration with flue gas scrubbing is possible for combustible packaging materials.

14. Transport information

14.1 UN Number

ADR/RID: UN1690 IMDG: UN1690
IATA:UN1690 DOT: UN1690

14.2 UN Proper Shipping Name

ADR/RID: SODIUM FLUORIDE, SOLID
IMDG: SODIUM FLUORIDE, SOLID
IATA: SODIUM FLUORIDE, SOLID
DOT: SODIUM FLUORIDE, SOLID

14.3 Transport hazard class(es)

ADR/RID: 6.1 IMDG: 6.1 IATA: 6.1 DOT:6.1

14.4 Packing group, if applicable

ADR/RID: III IMDG: III IATA: III DOT:III

14.5 Environmental hazards

ADR/RID: No IMDG: No IATA: No

14.6 Special Provisions

IB8,T1,TP33

14.7 Transport in bulk according to IMO instruments

no data available

15. Regulatory information

15.1 Safety, health and environmental regulations specific for the product in question

Chemical name	Common names and synonyms	CAS number	EC number
Sodium fluoride	Sodium Fluoride	7681-49-4	231-667-8
United States Toxic Substances Control Act (TSCA) Inventory			Listed.
California Prop. 65 Components			Not Listed.
New Jersey Right To Know - Right to Know Hazardous Substance List (RTKHSL)			Listed.
Massachusetts Right To Know - MASSACHUSETTS SUBSTANCE LIST (MSL)			Listed.
Pennsylvania Right To Know - HAZARDOUS SUBSTANCE LIST			Listed.
Federal Drinking Water Guidelines	no data available		
State Drinking Water Guidelines	no data available		
Clean Water Act Requirements	no data available		
CERCLA Reportable Quantities	no data available		
RCRA Requirements	no data available		
FIFRA Requirements	no data available		
FDA Requirements	no data available		

16. Other information

Information on revision

Creation Date Dec. 22, 2020
Revision Date Dec. 30, 2020

Abbreviations and acronyms

- CAS: Chemical Abstracts Service
- ADR: European Agreement concerning the International Carriage of Dangerous Goods by Road
- RID: Regulation concerning the International Carriage of Dangerous Goods by Rail
- IMDG: International Maritime Dangerous Goods
- IATA: International Air Transportation Association
- TWA: Time Weighted Average
- STEL: Short term exposure limit
- LC50: Lethal Concentration 50%
- LD50: Lethal Dose 50%
- EC50: Effective Concentration 50%

References

- IPCS - The International Chemical Safety Cards (ICSC), website: http://www.ilo.org/dyn/icsc/showcard.home
- HSDB - Hazardous Substances Data Bank, website: https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm
- IARC - International Agency for Research on Cancer, website: http://www.iarc.fr/
- eChemPortal - The Global Portal to Information on Chemical Substances by OECD, website: http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en
- CAMEO Chemicals, website: http://cameochemicals.noaa.gov/search/simple
- ChemIDplus, website: http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp
- ERG - Emergency Response Guidebook by U.S. Department of Transportation, website: http://www.phmsa.dot.gov/hazmat/library/erg
- Germany GESTIS-database on hazard substance, website: http://www.dguv.de/ifa/gestis/gestis-stoffdatenbank/index-2.jsp
- ECHA - European Chemicals Agency, website: https://echa.europa.eu/

Other Information

Specific treatment is necessary in case of poisoning with this substance; the appropriate means with instructions must be available.

Any questions regarding this SDS, Please send your inquiry to sds@xixisys.com

Disclaimer: The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. We as supplier shall not be held liable for any damage resulting from handling or from contact with the above product.

SHANGHAI MINTCHEM DEVELOPMENT CO., LTD

R201C HUANAN BUILDING, NO. 1988 DONGFANG ROAD, PUDONG ZONE, SHANGHAI, CHINA

CERTIFICATE OF ANALYSIS

NAME OF PRODUCT: Sodium Fluoride Coarse NSF-60
INVOICE NUMBER: YY2417017
BATCH NUMBER: 2417017
PO NO.:132319
DATE:19 Mar,2024

No.	Item	Standard (%)	Result (%)
1.	NaF purity	98.5min	98.51
2.	Sodium Carbonate	0.5max	0.03
3.	Silicon Dioxide	0.5max	0.48
4.	Sulphate	0.3max	0.04
5.	HF	0.1max	0.02
6.	H2O(moisture)	0.5max	0.35
7.	Heavy metal(As Pb)	0.004max	0.0009
8.	Available Fluoride	43.8min	44.57
9.	Water Insoluble matter	0.6max	0.24
10.	Turbidity	10.0max	7.03
Particle Size			
1.	-20 mesh	98 min	99.0
2.	+100 mesh	50 min	62.5
3.	-325 mesh	5 max	2.1

Physical Properties

Molecular Weight 42

Boiling Point 1695°C

Melting Point 995°C

Specific Gravity 2.5-2.6

Solubility in Water 4.1g/100g H2O

上海明太化工发展有限公司
Shanghai Mintchem Development Co., Ltd.

Authorized Signature

We hereby certify that this lot of material meet the standard.

Products meets ANSI/AWWA standard B701-18 and is NSF-60 Certified.

_____
From: Jennifer PHILLIPS
Sent: 6/17/2025 10:10:52 PM
To: DOH Secretary's Office
Subject: Immediate Action Needed: Restore Independent Vaccine Oversight & Protect Access in Washington

External Email

☐
Dear Governor Ferguson and Health Policy Leaders,

I am writing with urgency to request the immediate reconvening and expansion of the Western States Scientific Safety Review Workgroup and coordinated state action to maintain vaccine access and oversight in Washington.

We are already seeing the effects of recent politicization of federal health agencies—and Washington families are being harmed.

When the CDC recently came out with new guidance that indicates enrolled VFC providers are no longer required to stock COVID vaccines (if they have a plan in place to refer patients for the vaccine, which is not happening either). As a direct result, we are already seeing pediatric patients being "referred" into one of the few clinics who will vaccinate babies and small children who aren't their own patients. The Washington State Department of Health has followed suit, instead of asserting their own standards. Providers are now dropping COVID-19 vaccine access for children, and our state has no plan in place to counter this dangerous vacuum. This is just the beginning.

The possibility of further federal rollbacks—under pressure from anti-vaccine ideologues now taking power at HHS, CDC, and FDA—demands urgent, coordinated response across Washington.

☐ Here's what needs to happen

immediately:

1. Governor Ferguson & Executive Staff

* Reconvene the Western States Scientific Safety Review Workgroup, with authority to evaluate all vaccine changes—not just COVID vaccines.
* Issue a directive to DOH to immediately review and, where necessary, maintain a Washington-specific immunization schedule based on scientific consensus—not federal political interference.
* Convene an emergency cross-agency task force including DOH, HCA, OIC, SBOH, and legislative leads to prepare contingency plans for vaccine access and insurance

coverage.

2. Department of Health & State Board of Health

* Issue state guidance requiring all pediatric and primary care providers who are enrolled in the VFC program to continue offering and keeping state recommended vaccine in stock for the population they serve.
* Develop a Washington-specific vaccine recommendation platform, with independent review capacity if federal standards are compromised.
* Update procurement and distribution plans to ensure vaccine availability even without VFC support.

3. State Legislators on Health & Budget Committees

* Introduce emergency legislation or funding riders to ensure COVID-19 and other vulnerable vaccines are covered and accessible regardless of federal moves.
* Hold public hearings on the risks of federal interference and the need for Washington to protect independent scientific decision-making.
* Direct HCA and OIC to guarantee insurance and Medicaid coverage for all state recommended vaccines.

4. Insurance Commissioner, HCA, and Medicaid Agencies

* Proactively classify essential vaccines as covered services under both public and private insurance, regardless of CDC status.
* Prepare alternative purchasing and reimbursement structures for providers who lose VFC support but wish to continue vaccinating.

IT NEEDS TO HAPPEN NOW!

Washington State has both the legal authority and infrastructure to make independent vaccine policy decisions.

Under RCW 43.20.050 and WAC 246-105, the Washington State Board of Health is authorized to determine immunization requirements for school and child care entry.

The Washington State Department of Health (DOH) maintains a state-specific immunization schedule and has discretion to issue guidance to providers.

The Health Care Authority (HCA) can ensure Medicaid coverage of recommended vaccines regardless of federal VFC program changes, and the Office of the Insurance Commissioner (OIC) has the regulatory authority to mandate private insurance coverage for essential vaccines.

Together, these entities can act now to safeguard vaccine access and public health in Washington, even in the face of the anti science anti vaccine movement.

Washington’s history of vaccine leadership—especially during COVID—has been possible because we had independent review capacity and science-based leadership. Without fast action, we risk falling behind and exposing our children to preventable diseases.

We cannot wait for federal agencies to fall further into anti-science hands. We must act now to protect public health in our state.

Thank you for your leadership. Please respond swiftly with a plan to reconvene the workgroup and mobilize state agencies and legislative action to protect vaccine access and trust in science.

With urgency and resolve,

Jennifer Phillips

Washington State resident, voter and healthcare worker for nearly 20 years.

_____

From: tweetfamily@comcast.net
Sent: 8/15/2025 11:15:34 AM
To: DOH WSBOH
Cc:
Subject: WSBOH Aug 20 meeting, public comment on fluoridation chemicals added to water


attachments\7272B22336064CB6_image.png


attachments\CA7840E23F3B466B_Cascade Columbia.pdf

External Email

Attached see a Bill of Lading on page 1 for the fluoridation chemicals added to the water in the city of Camas. Please note the hazard warnings on page 2 from the vendor of fluoridation chemicals pumped into city water supplies in Camas, WA.

Public Comment submitted by Margaret Tweet, WA resident

CASCADE COLUMBIA DISTRIBUTION TERMS AND CONDITIONS OF SALE

In the event seller accepts this order and ships a portion or all of the product ordered as shown on the face of this shipping order, the following terms and conditions shall apply:

1. Buyer acknowledges that the material sold under this agreement is or may be hazardous to human health and to the environment, and Buyer assumes all risk and liability for the use of the material. Buyer shall familiarize itself and keep informed (without reliance on the Seller) with respect to any hazards to persons or property involved in handling and/or using the material and the containers in which such material is shipped. Buyer shall advise all its employees, contractors, agents and others who handle and use the material for the Buyer and shall take such action as is reasonably necessary to advise others, including without limitation the customers of the Buyer, who are foreseeably the ultimate users of the material, of suspected or proven hazards of the material.

2. Due to hazardous nature of the material sold hereunder it is recognized that Buyer purchases such materials solely at Buyer's risk and that in no event shall the Seller be liable for direct, indirect, incidental, consequential, special, punitive, exemplary or speculative damages. Buyer's exclusive remedy hereunder shall be the reimbursement of the purchase price of the material with respect to which any claim is made, whether such claim is in respect to material delivered or for non-delivery, and whether or not based on negligence, breach of warranty or strict liability in tort.

3. Buyer hereby assumes all risks and liabilities for the use, storage and handling of the material and agrees to indemnify and hold Seller harmless from all liability including claims by Buyer and any third parties, including without limitation Buyer's employees and customers, arising out of or related to, directly or indirectly, the use, storage or handling of the material and regardless of whether the material is used in combination with other articles or substances or in any manufacturing process.

4. It is expressly understood that any technical or safety advice furnished by Seller with reference to the material is supplied without consideration and Seller assumes no obligation or liability for the accuracy or sufficiency of advice given or results obtained, all such advice being given and accepted at Buyer's risk.

5. Seller warrants that the goods supplied hereunder shall conform to the description on the reverse side of this form and are of Seller's standard quality. Seller makes no other warranty of any kind whatsoever, express or implied. Seller expressly disclaims all implied warranties, including without limitation the warranties of merchantability or fitness for a particular purpose. If Seller breaches said warranties, then the limit of damages shall be as set forth above.

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PORTLAND PH: (503) 625-5293 FAX: (503) 625-4335
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B/L NO.
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CAMAS, WA 98607-2108
USA

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03414	none	1/28/2022	200	

SHIP DATE	SHIP VIA	FOB REMARK	TERMS
2/1/2022	CASCADE DELIVERY	DELIVERED	N3

MUNI

QUANTITY ORDERED	QUANTITY SHIPPED	QUANTITY BACKORDER	PACKAGING	HM	DESCRIPTION	NET WEIGHT	GROSS WEIGHT	FRT CLS
40	40	0	50#BAG 666200TI	X	UN1690,SODIUM FLUORIDE, SOLID 6.1, PGIII, ERG 154 SODIUM FLUORIDE - 50# BAG 40/PALLET 211019	2000 Lbs	2080 Lbs	
49	49	0	50#BAG 608210TI		SALT CARGILL DIAMOND EXTRA COARSE NATURALS 50# BAG 49/PALLET	2450 Lbs	2548 Lbs	
					Lot Number: 2021-12-30 Packaging: 50 # BAG Qty: 49			
					Total Weights (LBS):	**4450.00**	**4628.00**	

Driver Note: PHONE #360-834-3264,

Driver Note: CALL 1 HOUR AHEAD
ALLEN 360-690-5095 OR
BRANDON 360-921-2873

424.00.534.830.36 AN

SHIPPER'S CERTIFICATION This is to certify that the above named materials are properly classified, described, packaged, marked and labeled and are in proper condition for transportation according to the applicable regulations of the Department of Transportation.

Signature: Matt K

Carrier Name, if different

Drivers Signature | Date

Customer Signature

Customer Print Name | Date

Signature here by confirms receipt of quantity stated in order and agrees to comply with the terms set forth on the back of this Form.

LOAD INFO	PULLED BY:		CHECKED BY:	
UNLOAD TIME	ARRIVE:		DEPART:	
PLACARDS	OFFERED:		ON TRUCK:	

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2. Due to hazardous nature of the material sold hereunder it is recognized that Buyer purchases such materials solely at Buyer's risk and that in no event shall the Seller be liable for direct, indirect, incidental, consequential, special, punitive, exemplary or speculative damages. Buyer's exclusive remedy hereunder shall be the reimbursement of the purchase price of the material with respect to which any claim is made, whether such claim is in respect to material delivered or for non-delivery, and whether or not based on negligence, breach of warranty or strict liability in tort.

3. Buyer hereby assumes all risks and liabilities for the use, storage and handling of the material and agrees to indemnify and hold Seller harmless from all liability including claims by Buyer and any third parties, including without limitation Buyer's employees and customers, arising out of or related to, directly or indirectly, the use, storage or handling of the material and regardless of whether the material is used in combination with other articles or substances or in any manufacturing process.

4. It is expressly understood that any technical or safety advice furnished by Seller with reference to the material is supplied without consideration and Seller assumes no obligation or liability for the accuracy or sufficiency of advice given or results obtained, all such advice being given and accepted at Buyer's risk.

5. Seller warrants that the goods supplied hereunder shall conform to the description on the reverse side of this form and are of Seller's standard quality. Seller makes no other warranty of any kind whatsoever, express or implied. Seller expressly disclaims all implied warranties, including without limitation the warranties of merchantability or fitness for a particular purpose. If Seller breaches said warranties, then the limit of damages shall be as set forth above.

6. Buyer warrants that Buyer has reviewed all Federal, State, and local laws, rules and regulations regarding the transportation, storage and use of the materials sold, and Buyer agrees to abide by those laws, rules and regulations.

7. This contract is governed under the laws of the State of Washington. In the event of any conflict between the parties or in the event of litigation the venue will be Seattle, King County, Washington.

8. Seller retains ownership of all returnable containers. Buyer may use the containers only for the storage of original contents. Buyer shall return the containers to Seller empty and in good condition withing 90 days for the date of delivery. Buyer shall pay a deposit on all returnable containers. Seller shall credit the deposit, less a handling fee, to Buyer's account if Buyer returns the containers F.O.B. Seller's return point in good condition within 90 days. If not returned within 90 days, Seller may reject the containers and retain the full amount of the deposit.

9. Seller is not liable for nonperformance or delay in performance caused by circumstances beyond Seller's control ("Force Majeure Event"). A Force Majeure Event includes, without limitation, (a) acts of God, war, riots, fire, explosions, floods, strikes, lockouts, injunctions, accidents, product short supply, unforseseen shutdown of major sources of supply, breakage of machinery, or apparatus, or national emergency, (b) Seller's inability to obtain prices Seller deams in its discretion to be commercially reasonable, the Product, fuel, power, raw materials, labor, containers or transportation facilites, (c) the occurrence of any unforeseeable contingency making performance impracticable, or (d) compliance in good faith with any applicable governmental statute, regulation, or order. Any delivery so suspended shall be cancelled without liability, but these Terms and Conditions shall ortherwise remain unaffected. This section does not apply to payment obligations.

From: Bill Osmunson
Sent: 8/10/2025 5:35:17 PM
To: DOH WSBOH
Cc:
Subject: Public Comment Board of Health August 2025

attachments\6F967B133BD24879_WSBOH_Fluoridation_Jurisdiction_Letter.docx

External Email

Please see attached, submitted for the August 2025 Board Meeting.

Thank you,
Bill Osmunson DDS MPH

Bill Osmunson, DDS, MPH

10300 181st Ave SE

Issaquah, WA 98027

425-466-0100

Email currently unavailable

August 10, 2025

To:

Washington State Board of Health

PO Box 47990

Olympia, WA 98504-7990

Re: Jurisdictional Avoidance by Local Water Districts and Cities Regarding Fluoridation Safety and Efficacy — Board's Sole Duty Under RCW 43.20.050

For more than 80 years, community water fluoridation (CWF) has been promoted as "safe and effective," yet it has never undergone the rigorous safety and efficacy review required for any other ingestible drug under the U.S. Food and Drug Administration's Center for Drug Evaluation and Research (FDA CDER) standards. At the federal level, FDA and the Environmental Protection Agency (EPA) have historically deferred to one another, leaving CWF outside formal drug review. In Washington State, local water districts and cities similarly defer to the State Board of Health and the Department of Health to determine fluoridation safety and efficacy.

Under RCW 43.20.050(2)(a), the Board has the non-delegable duty to "adopt rules controlling public water supplies, to assure safe and reliable public drinking water and to protect the public health." While RCW 57.08.012 allows water districts to choose whether to fluoridate, that permissive authority does not replace or diminish the Board's legal responsibility to ensure safety.

The Limits of Local and Departmental Authority

The Department of Health employs capable professionals, but none are trained or authorized as drug regulatory approval experts. They do not operate under FDA's structured system for approving drugs—no dedicated review divisions, no good

manufacturing practice (GMP) inspections, no formal benefit–risk determinations, and no post-market safety surveillance.

Local governments and water utilities are even further removed from such expertise. City councils and district boards are not pharmacology or toxicology bodies and are not competent to evaluate complex medical safety data. Their reliance on the assumption that fluoridation has passed rigorous federal safety review is misplaced.

The Evidence Gap

FDA has never approved ingestible prescription fluoride "supplements" (drops, tablets, lozenges) for caries prevention. On July 23, 2025, the Regan Udall Foundation and FDA held a public listening session titled "Use of Orally Ingestible Unapproved Prescription Drug Products Containing Fluoride in the Pediatric Population." The meeting underscored that these products remain unapproved drugs, and no randomized controlled trials (RCTs) for either benefit or safety were presented by any participant.

The 2024 Cochrane Review again confirmed that no RCTs exist for the ingestion of fluoride—whether from supplements or water fluoridation—that meet modern FDA standards for efficacy and safety. The Washington Fluoridation Panel has not reviewed any high-quality developmental neurotoxicity studies, despite NIH-funded research (Bashash 2017; Green 2019; Till 2020) showing possible neurodevelopmental risks at current exposure levels.

Pharmacokinetics (PK): A Missing Cornerstone of Safety

For FDA approval, one of the first steps is a set of pharmacokinetic (PK) studies—plainly, research on what the body does with a substance after you swallow it. These studies answer:

1. Absorption – How much fluoride gets from the stomach into the blood, and how fast.
2. Distribution – Where fluoride travels in the body (bones, teeth, brain, kidneys, etc.).
3. Metabolism – Whether the body changes fluoride into other compounds.
4. Excretion – How quickly fluoride leaves the body, mainly through urine, and how much remains stored in bones or other tissues.
5. Half-life – How long it takes for fluoride levels in the body to drop by half after a dose.
6. Special populations – How infants, pregnant women, people with kidney disease, or those with genetic differences handle fluoride compared to healthy adults.

Without modern PK data, no one can set a precise, safe lifetime exposure for all people—especially the most vulnerable. For drugs, FDA always requires PK data before approval; for CWF, such comprehensive studies have never been done.

Statutory Duty and Ethical Obligation

The Board's statutory role is proactive: to assure safety before exposure, not after harm is shown. Endorsements from other agencies or professional associations cannot substitute for the Board's independent obligation under RCW 43.20.050.

From an ethical standpoint, the principle of non-maleficence ("first, do no harm") compels a cautious approach, especially for substances given without individual consent. Justice requires protecting infants, medically compromised individuals, and genetically susceptible people to the same standard as the healthiest adults.

A Call for Regulatory Courage

It is time for the Washington State Board of Health to:
1. Acknowledge that neither the Department of Health nor local utilities have the authority or expertise to approve drugs.
2. Recognize that CWF, as practiced, meets the legal definition of a drug under the FD&C Act and requires FDA CDER review.
3. Suspend state rules authorizing fluoridation until an FDA New Drug Application (NDA) is submitted, reviewed, and approved.
4. Formally petition FDA CDER to take jurisdiction over fluoridation chemicals used for systemic disease prevention.

Respectfully submitted,

Bill Osmunson, DDS, MPH

Retired Dentist & Public Health Professional

References

1. RCW 43.20.050 – Powers and duties of the state board of health.
2. RCW 57.08.012 – Powers of water districts regarding fluoridation.
3. Regan Udall Foundation/FDA. "Use of Orally Ingestible Unapproved Prescription Drug Products Containing Fluoride in the Pediatric Population." Public Listening Session, July 23, 2025.
4. Iheozor-Ejiofor Z, et al. Water fluoridation for the prevention of dental caries. Cochrane Database Syst Rev. 2024.
5. Bashash M, et al. Prenatal fluoride exposure and cognitive outcomes in children. Environ Health Perspect. 2017.
6. Green R, et al. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring. JAMA Pediatr. 2019.
7. Till C, et al. Fluoride exposure and attention deficit hyperactivity disorder in Canadian youth. Environ Int. 2020.

From: Lisa Brienen
Sent: 4/4/2025 10:26:07 PM
To: DOH WSBOH
Cc:
Subject: fluoridation of water needs to stop

External Email

Dear WA BOH,

"Recently, epidemiological studies have suggested that fluoride is a human developmental neurotoxicant that reduces measures of intelligence in children, placing it into the same category as toxic metals (lead, methylmercury, arsenic) and polychlorinated biphenyls. If true, this assessment would be highly relevant considering the widespread fluoridation of drinking water and the worldwide use of fluoride in oral hygiene products such as toothpaste."

I know you've already been provided with innumerable studies for your various meetings – related to the harms of fluoridation of water. But here is one more https://pmc.ncbi.nlm.nih.gov/articles/PMC3956646/ <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fpmc.ncbi.nlm.nih.gov%2Farticles%

Ultimately adding fluoride to the water supply will be stopped state by state. It would be ideal for the WA BOH to make the medically sound decision to stop the adding of a neurotoxin to the water supply now.

Sincerely, Lisa Brienen

From: Bill Osmunson
Sent: 8/11/2025 1:56:24 PM
To: DOH WSBOH
Cc:
Subject: Jurisdiction: Public Comment August 20



attachments\8F8DBD2D7C4E40D6_WSBOH_Fluoridation_Jurisdiction_Letter.docx

External Email

Greetings,
Attached is public comment for the Board of Health meeting in August.

Thank you,
Bill Osmunson DDS MPH
4254660100

Bill Osmunson, DDS, MPH

10300 181st Ave SE

Issaquah, WA 98027

425-466-0100

Email currently unavailable

August 10, 2025

To:

Washington State Board of Health

PO Box 47990

Olympia, WA 98504-7990

Re: Jurisdictional Avoidance by Local Water Districts and Cities Regarding Fluoridation Safety and Efficacy — Board's Sole Duty Under RCW 43.20.050

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1. Absorption – How much fluoride gets from the stomach into the blood, and how fast.
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3. Metabolism – Whether the body changes fluoride into other compounds.
4. Excretion – How quickly fluoride leaves the body, mainly through urine, and how much remains stored in bones or other tissues.
5. Half-life – How long it takes for fluoride levels in the body to drop by half after a dose.
6. Special populations – How infants, pregnant women, people with kidney disease, or those with genetic differences handle fluoride compared to healthy adults.

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Respectfully submitted,

Bill Osmunson, DDS, MPH

Retired Dentist & Public Health Professional

References

1. RCW 43.20.050 – Powers and duties of the state board of health.
2. RCW 57.08.012 – Powers of water districts regarding fluoridation.
3. Regan Udall Foundation/FDA. "Use of Orally Ingestible Unapproved Prescription Drug Products Containing Fluoride in the Pediatric Population." Public Listening Session, July 23, 2025.
4. Iheozor-Ejiofor Z, et al. Water fluoridation for the prevention of dental caries. Cochrane Database Syst Rev. 2024.
5. Bashash M, et al. Prenatal fluoride exposure and cognitive outcomes in children. Environ Health Perspect. 2017.
6. Green R, et al. Association between maternal fluoride exposure during pregnancy and IQ scores in offspring. JAMA Pediatr. 2019.
7. Till C, et al. Fluoride exposure and attention deficit hyperactivity disorder in Canadian youth. Environ Int. 2020.

From: bill teachingsmiles.com
Sent: 7/27/2025 6:44:20 PM
To: DOH WSBOH,Jenks, Lauren (DOH)
Cc:
Subject: Public Comment for August 2025

attachments\BBE07888A0114D9A_Fluoridation Safety Whitepaper.odt

External Email

Dear WSBOH,

I am sending this early so the Board has plenty of time to digest it.

And repeating, I am requesting 15 minutes at the next Board meeting to present an edited version specifically for Washington State of what I presented to the FDA CDER. The acceptance of the evidence by the Director of the CDER was amazing.

Pasted her and attached. The attached may come through with correct formatting.

Thank you,
Bill Osmunson DDS MPH

To: Washington State Board of Health
Public Comment for August 2025, Board of Health Meeting
From: Bill Osmunson, DDS, MPH
Subject: Legal and Ethical Obligations to Assure the Safety of Fluoridated Public Drinking Water
Date: July 27, 2025

Title: Assuring the Safety of Public Drinking Water: A Legal and Ethical Imperative Regarding Fluoridation

Introduction This paper examines the legal, ethical, and scientific basis for requiring a high degree of certainty regarding the safety of public drinking water, with a focus on fluoride added for the purpose of preventing dental caries. It explores relevant federal and state laws, regulatory frameworks, and ethical considerations that mandate a shift from evaluating the "certainty of harm" to demanding "certainty of safety."

1. Legal Mandates for Assuring Drinking Water Safety

1. Washington State Law: RCW 43.20.050(2) Washington State explicitly requires public health officials to assure drinking water safety. RCW 43.20.050(2) mandates:

"In order to protect public health, the state board of health shall: (a) Adopt rules as necessary to assure safe and reliable public drinking water." [1]
The statutory use of the word "assure" carries a high standard. The Oxford English Dictionary defines "assure" as "to tell someone something positively or confidently to dispel any doubts they may have." [2] In this context, public health agencies have a legal and ethical responsibility to ensure that drinking water is not only presumed safe, but demonstrably so—leaving no reasonable doubt.

2. Federal Law: Food, Drug, and Cosmetic Act (FDCA) Under the FDCA, a product intended for the prevention of disease is legally defined as a drug:

"The term 'drug' means ... (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals." (21 U.S.C. § 321(g)(1)(B)) [3]
When fluoride is added to drinking water with the intent to prevent dental caries, it meets this definition and is therefore subject to FDA regulation. However, no fluoride additive used for water fluoridation has been approved through the FDA's New Drug Application (NDA) process.

3. Supreme Court Precedent: Weinberger v. Hynson The U.S. Supreme Court has emphasized the necessity of affirmative proof of both safety and efficacy for drugs:

"Congress did not intend that a drug be approved for efficacy on the basis of belief in its benefits. Rather, Congress intended that efficacy be established by substantial evidence as a condition to approval." (Weinberger v. Hynson, Westcott & Dunning, Inc., 412 U.S. 609, 618 (1973)) [4]
This reinforces that absence of proven harm is not enough; substantial affirmative evidence of safety and effectiveness is required.

4. Ethical Considerations: From Certainty of Harm to Certainty of Safety

1. The Flawed Standard: Certainty of Harm

Much of the scientific discourse surrounding fluoridation has been focused on whether harm from fluoride ingestion can be conclusively demonstrated with certainty, as the EPA required for the National Research Council's review of fluoride in drinking water for the EPA. This framing is problematic because:

* The absence of total certainty of harm for the statistical mean, does not equate to evidence of safety.
* Many vulnerable subgroups (infants, elderly, immunocompromised) are underrepresented or excluded in safety studies.

2. The Correct Standard: Assuring Safety with Confidence Public health ethics and administrative law require a more precautionary and protective approach:

* For medications, patients can consent and weigh risks.
* For drinking water, exposure is involuntary and universal.

Therefore, the ethical and legal burden is to demonstrate that public water, with addition of the highly toxic hydrofluosilicic acid, is safe to consume without individualized medical supervision or dosage control.

1. Quantifying "Assurance" To fulfill the statutory obligation to "assure" safety, public health authorities must pursue a level of certainty that is as close as possible to 100% confidence in safety. Practically, this could mean tolerating less than a 1% probability of harm—a far higher standard than the statistical norm used in scientific hypothesis testing ($p<0.05$).

2. Regulatory Implications: Fluoride as an Unapproved Drug

Given that fluoride is used in drinking water with therapeutic intent, and given the absence of FDA approval:

* Fluoridated water constitutes the distribution of an unapproved drug and the FDA CDER has confirmed unapproved drugs are misbranded and illegal to market without FDA approval.
* The FDA's longstanding deferral of enforcement action does not negate the lack of legal compliance.
* State and local agencies relying on CDC recommendations are not legally exempt from FDA's regulatory framework for drugs.
* The WSDH fluoridation panel members were chosen to some extent for their promotion and support for fluoridation. A paradigm change was too traumatic for them, not unlike asking the tobacco lobbyists to confirm tobacco is not safe. They desired absolute certainty fluoridation was causing serious harm, exceeding alleged benefit. However, they did not provide substantial evidence of efficacy with even a single randomized controlled trial that the ingestion of fluoride is effective, that the FDA CDER requires for approval. Without substantial evidence of a dosage for efficacy, determining harm or safety at that dosage is not possible.
*

Weighing the balance of benefit vs risk is not possible without substantial evidence of benefit. And further, the law does not charge the Board of Health with weighing the benefit vs the risk, rather only with determining safety. Benefit is under the jurisdiction of the only Federal Drug Regulatory Agency in the USA.
* The Board must give individuals the liberty to not have oppressive restrictions imposed on them when alternatives are cheaper and readily available: i.e. eating none organic foods, mechanically deboned meet, swallowing a pea size of fluoride toothpaste, etc.
* 60% to 70% of children show a biomarker of excess fluoride ingestion. Too many are ingesting too much fluoride.
* In court sworn depositions, neither the CDC, EPA, NSF, nor FDA had a single study reporting the dosage of fluoride which is safe to ingest, and neither does the Washington State Department of Health nor Board of Health have such a study.

3. Conclusion

Regulators must move beyond traditional harm-based frameworks and adopt the more rigorous standard required by law: certainty of safety. Washington State law (RCW 43.20.050) and the FDCA both demand more than the mere absence of evidence of harm—they require the confident dispelling of public doubt through affirmative proof of safety.
For an exposure as universal and essential as public drinking water, especially when it involves the administration of a pharmacologically active substance like fluoride, nothing short of near-absolute assurance of safety is ethically and legally acceptable.

4. References

1. Revised Code of Washington 43.20.050(2), State Board of Health—Rules. Available at: https://apps.leg.wa.gov/rcw/default.aspx?cite=43.20.050
2. Oxford English Dictionary. Definition of "assure."
3. 21 U.S.C. § 321(g)(1)(B), Federal Food, Drug, and Cosmetic Act.
4. Weinberger v. Hynson, Westcott & Dunning, Inc., 412 U.S. 609 (1973).
5. U.S. Food and Drug Administration. Drug Approval Process. https://www.fda.gov/drugs/drug-approvalsand-databases/drug-approvals
6. CDC. Community Water Fluoridation. https://www.cdc.gov/fluoridation/index.html

I. To: Washington State Board of Health

Public Comment for August 2025, Board of Health Meeting

II. From: Bill Osmunson, DDS, MPH

III. Subject: Legal and Ethical Obligations to Assure the Safety of Fluoridated Public Drinking Water

IV. Date: July 27, 2025

V. Title: Assuring the Safety of Public Drinking Water: A Legal and Ethical Imperative Regarding Fluoridation

Introduction This paper examines the legal, ethical, and scientific basis for requiring a high degree of certainty regarding the safety of public drinking water, with a focus on fluoride added for the purpose of preventing dental caries. It explores relevant federal and state laws, regulatory frameworks, and ethical considerations that mandate a shift from evaluating the "certainty of harm" to demanding "certainty of safety."

VI. Legal Mandates for Assuring Drinking Water Safety

A. Washington State Law: RCW 43.20.050(2) Washington State explicitly requires public health officials to assure drinking water safety. RCW 43.20.050(2) mandates:

> "In order to protect public health, the state board of health shall: (a) Adopt rules as necessary to assure safe and reliable public drinking water." [1]

The statutory use of the word "assure" carries a high standard. The Oxford English Dictionary defines "assure" as "to tell someone something positively or confidently to dispel any doubts they may have." [2] In this context, public health agencies have a legal and ethical responsibility to ensure that drinking water is not only presumed safe, but demonstrably so—leaving no reasonable doubt.

B. Federal Law: Food, Drug, and Cosmetic Act (FDCA) Under the FDCA, a product intended for the prevention of disease is legally defined as a **drug**:

> "The term 'drug' means ... (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals." (21 U.S.C. § 321(g)(1)(B)) [3]

When fluoride is added to drinking water with the intent to prevent dental caries, it meets this definition and is therefore subject to FDA regulation. However, **no fluoride additive used for water fluoridation has been approved through the FDA's New Drug Application (NDA) process**.

C. Supreme Court Precedent: Weinberger v. Hynson The U.S. Supreme Court has emphasized the necessity of affirmative proof of both safety and efficacy for drugs:

> "Congress did not intend that a drug be approved for efficacy on the basis of belief in its benefits. Rather, Congress intended that efficacy be established by substantial evidence as a condition to approval." (*Weinberger v. Hynson, Westcott & Dunning, Inc.*, 412 U.S. 609, 618 (1973)) [4]

This reinforces that absence of proven harm is not enough; substantial affirmative evidence of safety and effectiveness is required.

VII. Ethical Considerations: From Certainty of Harm to Certainty of Safety

A. The Flawed Standard: Certainty of Harm

Much of the scientific discourse surrounding fluoridation has been focused on whether harm from fluoride ingestion can be conclusively demonstrated with certainty, as the EPA required for the National Research Council's review of fluoride in drinking water for the EPA. This framing is problematic because:

- The absence of total certainty of harm for the statistical mean, does not equate to evidence of safety.
- Many vulnerable subgroups (infants, elderly, immunocompromised) are underrepresented or excluded in safety studies.

B. The Correct Standard: Assuring Safety with Confidence Public health ethics and administrative law require a more precautionary and protective approach:

- For medications, patients can consent and weigh risks.
- For drinking water, exposure is **involuntary** and **universal**.

Therefore, the ethical and legal burden is to demonstrate that public water, with addition of the highly toxic hydrofluosilicic acid, is **safe to consume without individualized medical supervision or dosage control**.

C. Quantifying "Assurance" To fulfill the statutory obligation to "assure" safety, public health authorities must pursue a level of certainty that is as close as possible to **100% confidence in safety**. Practically, this could mean tolerating less than a **1% probability of harm**—a far higher standard than the statistical norm used in scientific hypothesis testing ($p<0.05$).

VIII. Regulatory Implications: Fluoride as an Unapproved Drug

Given that fluoride is used in drinking water with therapeutic intent, and given the absence of FDA approval:

- Fluoridated water constitutes the **distribution of an unapproved drug and the FDA CDER has confirmed unapproved drugs are misbranded and illegal to market without FDA approval**.
- The FDA's longstanding deferral of enforcement action does not negate the lack of legal compliance.
- State and local agencies relying on CDC recommendations are not legally exempt from FDA's regulatory framework for drugs.
- The WSDH fluoridation panel members were chosen to some extent for their promotion and support for fluoridation. A paradigm change was too traumatic for them, not unlike asking the tobacco lobbyists to confirm tobacco is not safe. They desired absolute certainty fluoridation was causing serious harm, exceeding alleged benefit. However, they did

not provide substantial evidence of efficacy with even a single randomized controlled trial that the ingestion of fluoride is effective, that the FDA CDER requires for approval. Without substantial evidence of a dosage for efficacy, determining harm or safety at that dosage is not possible.

- Weighing the balance of benefit vs risk is not possible without substantial evidence of benefit. And further, the law does not charge the Board of Health with weighing the benefit vs the risk, rather only with determining safety. Benefit is under the jurisdiction of the only Federal Drug Regulatory Agency in the USA.
- The Board must give individuals the liberty to not have oppressive restrictions imposed on them when alternatives are cheaper and readily available: i.e. eating none organic foods, mechanically deboned meet, swallowing a pea size of fluoride toothpaste, etc.
- 60% to 70% of children show a biomarker of excess fluoride ingestion. Too many are ingesting too much fluoride.
- In court sworn depositions, neither the CDC, EPA, NSF, nor FDA had a single study reporting the dosage of fluoride which is safe to ingest, and neither does the Washington State Department of Health nor Board of Health have such a study.

IX. Conclusion

Regulators must move beyond traditional harm-based frameworks and adopt the more rigorous standard required by law: **certainty of safety**. Washington State law (RCW 43.20.050) and the FDCA both demand more than the mere absence of evidence of harm—they require the **confident dispelling of public doubt** through affirmative proof of safety.

For an exposure as universal and essential as public drinking water, especially when it involves the administration of a pharmacologically active substance like fluoride, **nothing short of near-absolute assurance of safety is ethically and legally acceptable**.

X. References

[1] Revised Code of Washington 43.20.050(2), State Board of Health—Rules. Available at: https://apps.leg.wa.gov/rcw/default.aspx?cite=43.20.050

[2] Oxford English Dictionary. Definition of “assure.”

[3] 21 U.S.C. § 321(g)(1)(B), Federal Food, Drug, and Cosmetic Act.

[4] *Weinberger v. Hynson, Westcott & Dunning, Inc.*, 412 U.S. 609 (1973).

[5] U.S. Food and Drug Administration. Drug Approval Process. https://www.fda.gov/drugs/drug-approvalsand-databases/drug-approvals

[6] CDC. Community Water Fluoridation. https://www.cdc.gov/fluoridation/index.html

From: Laurie Layne
Sent: 8/14/2025 8:32:09 PM
To: DOH WSBOH
Cc:
Subject: Public Comment

External Email

Hi I'm against fluoride being put in public drinking water . Such health issues as calcified pituitary glands and lower I Q scores concern me! As I'm sure it does parents of children and Teenagers! Furthermore our current US Department of Health and Human Services Secretary Robert F Kennedy has concerns also. Voicing his opposition to CDC recommending fluoridation of community drinking water referring to it as a dangerous neurotoxin. That should give us all pause. Time to follow the state of Utah and just say no!

From: shellies4@netzero.com
Sent: 8/8/2025 4:03:50 PM
To: DOH WSBOH
Cc:
Subject: Re public comments

External Email

Regards to the upcoming public meeting.

Dear Board,
Thank you for all your hard work!
Regards to the upcoming public meeting and public comments.
We DO NOT WANT FLOURIDE in our water system!
The people of the state of Washington have VOTED against it on 3 SEPARATE occasions!
We DO NOT WANT IT!!
Please STOP trying to pass it.
WE THE PEOPLE have voted against it over and over and over!
There are MANY studies that show LOTS of negative side effects and WE DONT WANT IT!!

How many times do we have to say so??
Is there a way to permanently remove it from agenda topics?
Does a group need to get together and sue the health department to make y'all stop??
What is it going to take?
Thank you for all your time and effort!

From: bill teachingsmiles.com
Sent: 6/23/2025 12:24:18 PM
To: DOH WSBOH,Kwan-Gett, Tao (DOH),Roberts, Michele (DOH),Duber, Herbie (DOH),Mentzer, Kari (DOH),Marcinkevage, Jessica (DOH),Dennis, Derrick (DOH),Christie, Emerson C (DOH),Bauman, Shay (SBOH),Locke, Tom /Tribe (DOHi),Phuc Ha,Berry, Allison 2 (DOHi),shawn.magee,Arndt, Amber R (DOH),Johnson, Laura W (DOH)
Subject: Paradoxical effect of fluoride increasing caries

attachments\CE485215F4D74135_Pardoxical effects of CWF Osmunson 6 25.pdf

External Email

To: Washington State Board of Health and Fluoridation Panel
From: Bill Osmunson, DDS, MPH
Email: bill@teachingsmiles.com
Phone: (425) 466-0100
Subject: Formal Comment Opposing Systemic Fluoride Ingestion via Community Water Fluoridation
Date: June 23, 2025

Re: Fluoridation Policy and Unintended Harms to Low-Income Populations
Dear Members of the Washington State Board of Health and Fluoridation Panel,
I respectfully submit this formal comment urging the Board to reconsider and suspend support for systemic fluoride ingestion via community water fluoridation in Washington State. Although intended to reduce dental caries in underserved populations, current scientific evidence shows that fluoridation may produce neurodevelopmental harm, reduce cognitive and economic potential, and ultimately increase dental caries among the poor—the very population it is intended to protect.

1. Fluoride Ingestion Is Linked to Neurodevelopmental Harm
Modern, high-quality studies show that fluoride exposure during pregnancy and early childhood is associated with reduced IQ and cognitive deficits:

* Green et al. (2019, JAMA Pediatrics) found that maternal urinary fluoride levels were associated with lower IQ in male offspring in a Canadian population with typical water fluoridation levels.
* Till et al. (2020, Environmental International) found that a 0.5 mg/L increase in water fluoride was associated with a 3.7-point decrease in IQ.
* Bashash et al. (2017, Environmental Health Perspectives) linked maternal urinary fluoride in Mexico to a 5–6 point drop in child IQ.
* The National Toxicology Program (NTP) 2020 Monograph, reviewed and supported by the National Academies of Sciences, Engineering, and Medicine (NASEM 2023), concluded that fluoride is "presumed to be a cognitive neurodevelopmental hazard to humans."

These findings are based on NIH- and Health Canada-funded studies with rigorous controls for confounding variables and are applicable to current U.S. fluoride exposure levels.

2. IQ Loss Leads to Lower Lifetime Income and Greater Poverty
The link between IQ and lifetime socioeconomic outcomes is well established:

* Grosse et al. (2002) estimated that each IQ point lost reduces lifetime income by approximately 1.76%.
* Population-wide reductions in cognitive potential caused by early fluoride exposure

could result in billions of dollars in lost productivity annually (Trasande et al., 2005; Osmunson et al., 2025).

This means that fluoride ingestion may push children into lower income brackets, increasing poverty and compounding disadvantage.

3. Poverty Is the Primary Risk Factor for Dental Caries
Research shows that poverty—not fluoridation status—is the strongest predictor of untreated dental caries:

* Vargas et al. (1998, J Dent Res) found that low-income children had more decay regardless of water fluoridation.
* CDC NHANES data (2011–2016) show that dental decay prevalence remains highest among low-income children in both fluoridated and non-fluoridated areas.

Thus, when fluoride ingestion impairs neurodevelopment and economic potential, it indirectly raises the risk of future dental caries.

4. Fluoridation May Increase, Not Decrease, Long-Term Caries Risk
By lowering IQ and income—two major predictors of lifetime oral health—fluoridation may inadvertently increase dental caries risk, especially among the poor. This creates a self-reinforcing loop in which the "solution" worsens the problem:

A[Systemic Fluoride Ingestion] --> B[Neurodevelopmental Harm / IQ Loss]
B --> C[Lower Educational Achievement]
C --> D[Lower Lifetime Earnings]
D --> E[Increased Poverty]
E --> F[Higher Risk of Dental Caries]
F --> G[Increased Pressure for Fluoridation]
This cycle directly contradicts the goals of public health, especially when viewed through a health equity lens.

5. Evidence for Fluoridation’s Caries Benefit Is Weak in Modern Contexts
The often-cited 24% reduction in dental caries is based on outdated studies reviewed in the 2015 Cochrane Review, which itself noted the high risk of bias and lack of modern data. The updated 2024 Cochrane Review and more recent studies reflect the limited benefit of systemic fluoridation in populations already using fluoride toothpaste:

* Iheozor-Ejiofor et al., Cochrane Oral Health Group (2024 update) concluded that:
  “The evidence on the effectiveness of water fluoridation in current populations is limited in quantity and quality. Most studies were conducted before the widespread use of fluoride toothpaste.”
  “We found insufficient evidence that fluoridation reduces caries in the primary dentition of children under age 5.”

In short, any marginal benefit must be weighed against the far more serious and long-lasting neurodevelopmental harms.

6. Legal and Ethical Issues: FDA Has Not Approved Fluoridated Water as Safe or Effective

Under RCW 69.38.010, fluoride intended for the prevention of disease meets the definition of a legend drug. Yet:

* The FDA has never approved fluoride for ingestion via drinking water;
* The EPA regulates only contaminant levels, not safety or benefit of purposeful ingestion;

* No federal agency has conducted a drug-level review of the safety and efficacy of fluoride in water.

As a result, Washington State is supporting the addition of an unapproved drug to public water supplies without informed consent or clinical oversight, raising serious questions of legal authority, ethical responsibility, and public trust.

Conclusion and Recommendations
Given the current state of science, I urge the Washington State Board of Health to:

1. Acknowledge the scientifically credible evidence that fluoride is a neurotoxicant at levels common in fluoridated communities;
2. Reassess the net population-level effect of fluoridation on dental caries, especially in the context of socioeconomic harm;
3. Call for an immediate moratorium on expanding and continuing fluoridation programs until the FDA properly evaluates fluoride for ingestion as a drug;
4. Prioritize ethical standards, including informed consent and environmental justice, especially for low-income and marginalized communities.

Fluoridation is no longer a simple dental policy—it is a complex public health, ethical, and legal issue with far-reaching consequences.
Respectfully submitted,
Bill Osmunson, DDS, MPH
bill@teachingsmiles.com
(425) 466-0100

To: Washington State Board of Health
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Thus, when fluoride ingestion impairs neurodevelopment and economic potential, it **indirectly raises the risk of future dental caries**.

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  "We found insufficient evidence that fluoridation reduces caries in the primary dentition of children under age 5."

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Fluoridation is no longer a simple dental policy—it is a complex public health, ethical, and legal issue with far-reaching consequences.

Respectfully submitted,
Bill Osmunson, DDS, MPH
bill@teachingsmiles.com
(425) 466-0100

From: Roberts, Michele (DOH)
Sent: 6/27/2025 11:01:03 AM
To: Jennifer PHILLIPS,DOH Secretary's Office
Subject: RE: Immediate Action Needed: Restore Independent Vaccine Oversight & Protect Access in Washington


attachments\4FE810DE4B664EC3_image001.png


attachments\DCC39F8503404189_image002.png

Dear Jennifer,

Thank you for contacting us with your questions and concerns about COVID-19 vaccine availability, vaccine oversight and protecting vaccine access in Washington. I'm responding on behalf of the Department of Health. We appreciate your engagement in public health and your interest in protecting communities across Washington.

The Washington State Department of Health (DOH) continues to recommend that everyone 6 months and older, including pregnant people, receive the current COVID-19 vaccine. This guidance is informed by the Washington State Vaccine Advisory Committee (VAC) <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoh.wa.gov%2Fpublic-health-provider-resources%2Fpublic-health-system-resources-and-services%2Fimmunization%2Fvaccine-advisory-committee-vac&data=05%7C02%7CWSBOH%40SBOH.WA.GOV%7Ce4b38f04d8e2432c643508ddb5a492fa%7C11d0e. The VAC serves as an advisory body to the Department on appropriate recommendations to control vaccine preventable diseases in Washington State.

We are working hard behind the scenes and closely monitoring federal updates and reviewing data from the CDC and FDA to ensure our state's recommendations reflect the latest science. As changes arise, we are committed to promptly sharing updates with the public and our partners.

Our goal is to ensure vaccine access remains safe, equitable, and available to all Washington residents, especially those at higher risk of severe illness. We also support health care providers in offering up-to-date guidance and resources.

You can stay informed and help by reading and sharing these resources:

* COVID-19 Vaccine Information | DOH <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoh.wa.gov%2Fyou-and-your-family%2Fimmunization%2Fdiseases-and-vaccines%2Fcovid-19&data=05%7C02%7CWSBOH%40SBOH.WA.GOV%7Ce4b38f04d8e2432c643508ddb5a492fa%7C11d0e2

* COVID-19 Vaccine Information for Health Care Providers | DOH <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoh.wa.gov%2Fpublic-health-provider-resources%2Fpublic-health-system-resources-and-services%2Fimmunization%2Fcovid-19-information&data=05%7C02%7CWSBOH%40SBOH.WA.GOV%7Ce4b38f04d8e2432c643508ddb5a492fa%7

* 2024–2025 COVID-19 Vaccine Immunization Schedule for People 6 Months of Age and Older <https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.cdc.gov%2Fvaccines%2Fcov19%2Fdownloads%2FCOVID-19-immunization-schedule-ages-6months-older.pdf&data=05%7C02%7CWSBOH%40SBOH.WA.GOV%7Ce4b38f04d8e2432c643508ddb5a492fa%7C1

*

Vaccines remain one of the most effective tools we have to prevent serious illness, reduce hospitalizations, and protect our communities. Thank you again for reaching out.

Michele

P.S. I sometimes work flexible hours, so while it suits me to email now, I don't expect a response or action outside of your own working hours.

Michele Roberts, MPH, MCHES

Gender Pronouns: She/Her

Assistant Secretary

Prevention and Community Health Division

Washington State Department of Health

Michele.Roberts@doh.wa.gov <mailto:Michele.Roberts@doh.wa.gov>

360-791-6724 | www.doh.wa.gov <https://gcc02.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.doh.wa.gov%2F&data=05%7

<https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdoh.wa.gov%2Fabout-us%2Fcontact-us%2Fsocial-media&data=05%7C02%7CWSBOH%40SBOH.WA.GOV%7Ce4b38f04d8e2432c643508ddb5a492fa%7C11d

From: Jennifer PHILLIPS <pinupgirl1976@msn.com>
Sent: Tuesday, June 17, 2025 10:11 PM
To: DOH Secretary's Office <DOH.Secretary@DOH.WA.GOV>
Cc: Bays, Nathan (GOV) <Nathan.Bays@GOV.wa.gov>; Gaul, Judy (GOV) <Judy.Gaul@GOV.wa.gov>; Minor, Rose (GOV) <Rose.Minor@GOV.wa.gov>; Albert, Carol (GOV) <Carol.Albert@GOV.wa.gov>; Frockt, David (GOV) <David.Frockt@GOV.wa.gov>; Safford, Caitlin (GOV) <Caitlin.Safford@GOV.wa.gov>; Fathi, Sahar (GOV) <Sahar.Fathi@GOV.wa.gov>; DOH WSBOH <WSBOH@SBOH.WA.GOV>; healthandwellness@sboh.wa.gov; sarah.dejong@snoco.org;

KCBOHAdmin@kingcounty.gov; DOH Information <DOH.Information@DOH.WA.GOV>; DOH Secretary's Office <DOH.Secretary@DOH.WA.GOV>; DOH Office of the Chief of Staff <COS@doh.wa.gov>; DOH Office of Prevention Safety and Health <OPSH@doh.wa.gov>; DOH Office of Health and Science <OHS@doh.wa.gov>; DOH OS Executive Office of Policy Planning and Evaluation <OPPE@doh.wa.gov>; Salehi, Atousa (DOH) <Atousa.Salehi@doh.wa.gov>; DOH Office of Immunization <OI@doh.wa.gov>; DOH PCH OI WA Childhood Vaccines <WAChildhoodVaccines@DOH.WA.GOV>; Roberts, Michele (DOH) <Michele.Roberts@doh.wa.gov>; Sherls, Jamilia J (DOH) <Jamilia.Sherls@doh.wa.gov>; Christensen, Allen T (DOH) <Allen.Christensen@doh.wa.gov>; Lindeblad, MaryAnne (HCA) <maryanne.lindeblad@hca.wa.gov>; Zerzan, Judy (HCA) <judy.zerzan@hca.wa.gov>; Linke, Taylor C (HCA) <taylor.linke@hca.wa.gov>; Fotinos, Charissa (HCA) <charissa.fotinos@hca.wa.gov>; Klein, Evan (HCA) <evan.klein@hca.wa.gov>; O'Neill, Shawn (HCA) <shawn.oneill@hca.wa.gov>; OIC Policy <policy@oic.wa.gov>; OIC DL Public Affairs <OICDLPublicAffairs@oic.wa.gov>; info@wavaccine.org; info@wcaap.org; wsma@wsma.org; Cleveland, Annette (WA Senate) <annette.cleveland@leg.wa.gov>; Robinson, June <june.robinson@leg.wa.gov>; Dhingra, Manka (LEG) <Manka.Dhingra@leg.wa.gov>; Bronoske, Dan (LEG) <Dan.Bronoske@leg.wa.gov>; Davis, Lauren (LEG) <Lauren.Davis@leg.wa.gov>; Macri, Nicole (LEG) <nicole.macri@leg.wa.gov>; Simmons, Tarra <Tarra.Simmons@leg.wa.gov>; Stonier, Monica <monica.stonier@leg.wa.gov>; Thai, My-Linh <My-Linh.Thai@leg.wa.gov>
Subject: Immediate Action Needed: Restore Independent Vaccine Oversight & Protect Access in Washington

External Email

☐

Dear Governor Ferguson and Health Policy Leaders,

I am writing with urgency to request the immediate reconvening and expansion of the Western States Scientific Safety Review Workgroup and coordinated state action to maintain vaccine access and oversight in Washington.

We are already seeing the effects of recent politicization of federal health agencies—and Washington families are being harmed.

When the CDC recently came out with new guidance that indicates enrolled VFC providers are no longer required to stock COVID vaccines (if they have a plan in place to refer patients for the vaccine, which is not happening either). As a direct result, we are already seeing pediatric patients being "referred" into one of the few clinics who will vaccinate babies and small children who aren't their own patients. The Washington State Department of Health has followed suit, instead of asserting their own standards. Providers are now dropping COVID-19 vaccine access for children, and our state has no plan in place to counter this dangerous vacuum. This is just the beginning.

The possibility of further federal rollbacks—under pressure from anti-vaccine ideologues now taking power at HHS, CDC, and FDA—demands urgent, coordinated response across Washington.

☐ Here’s what needs to happen

immediately:

1. Governor Ferguson & Executive Staff

* Reconvene the Western States Scientific Safety Review Workgroup, with authority to evaluate all vaccine changes—not just COVID vaccines.
* Issue a directive to DOH to immediately review and, where necessary, maintain a Washington-specific immunization schedule based on scientific consensus—not federal political interference.
* Convene an emergency cross-agency task force including DOH, HCA, OIC, SBOH, and legislative leads to prepare contingency plans for vaccine access and insurance coverage.

2. Department of Health & State Board of Health

* Issue state guidance requiring all pediatric and primary care providers who are enrolled in the VFC program to continue offering and keeping state recommended vaccine in stock for the population they serve.
* Develop a Washington-specific vaccine recommendation platform, with independent review capacity if federal standards are compromised.
* Update procurement and distribution plans to ensure vaccine availability even without VFC support.

3. State Legislators on Health & Budget Committees

* Introduce emergency legislation or funding riders to ensure COVID-19 and other vulnerable vaccines are covered and accessible regardless of federal moves.
* Hold public hearings on the risks of federal interference and the need for Washington to protect independent scientific decision-making.
* Direct HCA and OIC to guarantee insurance and Medicaid coverage for all state recommended vaccines.

4. Insurance Commissioner, HCA, and Medicaid Agencies

* Proactively classify essential vaccines as covered services under both public and private insurance, regardless of CDC status.
* Prepare alternative purchasing and reimbursement structures for providers who lose VFC support but wish to continue vaccinating.

IT NEEDS TO HAPPEN NOW!

Washington State has both the legal authority and infrastructure to make independent vaccine policy decisions.

Under RCW 43.20.050 and WAC 246-105, the Washington State Board of Health is authorized to determine immunization requirements for school and child care entry.

The Washington State Department of Health (DOH) maintains a state-specific immunization schedule and has discretion to issue guidance to providers.

The Health Care Authority (HCA) can ensure Medicaid coverage of recommended vaccines regardless of federal VFC program changes, and the Office of the Insurance Commissioner (OIC) has the regulatory authority to mandate private insurance coverage for essential vaccines.

Together, these entities can act now to safeguard vaccine access and public health in Washington, even in the face of the anti science anti vaccine movement.

Washington's history of vaccine leadership—especially during COVID—has been possible because we had independent review capacity and science-based leadership. Without fast action, we risk falling behind and exposing our children to preventable diseases.

We cannot wait for federal agencies to fall further into anti-science hands. We must act now to protect public health in our state.

Thank you for your leadership. Please respond swiftly with a plan to reconvene the workgroup and mobilize state agencies and legislative action to protect vaccine access and trust in science.

With urgency and resolve,

Jennifer Phillips

Washington State resident, voter and healthcare worker for nearly 20 years.

_____

From: Bob Lutz
Sent: 8/15/2025 9:01:43 AM
To: DOH WSBOH
Cc:
Subject: My Public Comments concerning the petition to remove chemicals from water to prevent disease



attachments\EACCDEA365B24483_WSBOH Fluoride letter final.docx

External Email

August 14, 2025

TO: Washington State Board of Health

FROM: Jim Sledge, DDS and Bob Lutz, MD MPH

SUBJ: Community Water Fluoridation

Every few years, it seems there is a new attack on community water fluoridation. We, in the dental and public health sectors, must again remind those in governmental agencies of the effectiveness and safety of fluoride. For nearly 80 years, communities and water districts throughout the United States have embraced community water fluoridation as a valuable health measure that has profoundly improved the oral, and overall health for millions of people. Research has consistently demonstrated the benefits of fluoride for reducing the incidence of tooth decay. "The U.S. Public Health Service; the United Kingdom's National Institute for Health Research, Centre for Reviews and Dissemination, at the University of York; and the National Health and Medical Research Council, Australia have all conducted scientific reviews by expert panels and concluded that community water fluoridation is a safe and effective way to promote good oral health and prevent decay," according to a May 2024 CDC Scientific Statement on Community Water Fluoridation.

Fluoride is a naturally occurring mineral found in soil and water. Almost all natural sources of water contain some amount of fluoride, but often at levels inadequate to prevent tooth decay. Community water fluoridation is the process of adjusting the amount of fluoride in drinking water to the recommended national level of 0.7 milligrams of fluoride per liter of water. The CDC has stated "Community water fluoridation is a cornerstone strategy for prevention of cavities in the U.S. It is a practical, cost-effective, and equitable way for communities to improve their residents' oral health regardless of age, education, or income." It also identified it as one of the 10 great public health interventions of the 20th century because of the dramatic decline in cavities since community water fluoridation started in 1945.

Community water fluoridation is cost-effective, estimated less than $1 per person per year in the United States, and the return on that investment is substantial, with studies estimating that every $1 spent on water fluoridation saves about $20 to $50 in dental treatment costs. A recent micro-simulation model of oral health outcomes predicted removing fluoride from the US public water supply would result in over 25 million additional decayed teeth in children within five years, costing an estimated $9.8 billion in additional dental care expenses. This impact would be particularly severe for children on Medicaid or those without insurance, of significant concern given projected decreases in access to Medicaid coverage.

There is evidence and research to demonstrate the effects of eliminating community water fluoridation. Juneau, Alaska voted to remove fluoride in 2007, leading to a rise in cavity-related procedures and costs, according to a 2018 study. And Calgary, Alberta, Canada did likewise in 2011 but decided to restore it in 2021 due to negative oral health outcomes. A study examining

the impact of fluoridation cessation on 3-5-year-old children in Israel found a statistically significant increase in the mean number of dental treatments, including restorations and crowns, after fluoridation was stopped.

Opponents of community water fluoridation increasingly cite research demonstrating an association of maternal consumption of fluoridated water and neurocognitive and behavioral issues of children. A recent Systematic Review and Meta-Analysis concluded there were "inverse associations and a dose-response association between fluoride measurements in urine and drinking water and children's IQ across the large multi-country epidemiological literature. There were limited data and uncertainty in the dose-response association between fluoride exposure and children's IQ when fluoride exposure was estimated by drinking water alone at concentrations less than 1.5 mg/L." It is important to note fluoride levels in the studies analyzed, which were all conducted overseas, were at or above 1.5 milligrams per liter, more than double the amount in US water systems. While the research on lower levels of fluoride and neurodevelopment is more limited, recent studies out of the US and Australia show that fluoride at the levels found in US water systems is not associated with lower IQ in children.

The National Toxicology Program (NTP) Review of Fluoride Exposure, Neurodevelopment, Cognitive Health Effects (2024) has further raised concerns of this association and utilized by opponents of water fluoridation. It, however, concluded there is no evidence of a causal relationship or biological mechanism for fluoride's effects on cognitive development and acknowledged the weakness of the evidence suggesting such. Nor did it find harm associated with the current recommended levels for community water fluoridation. Finally, it did not address the benefits of community water fluoridation.

A Cochrane report released in the fall of 2024 has been cited by opponents as evidence against community water fluoridation. While noting findings from research prior to 1975 were more robust and the current benefits of community water fluoridation may be less than those prior to the addition of fluoride to toothpaste and other available sources of fluoride, it nevertheless found adding fluoride to water supplies may lead to slightly less tooth decay in children's baby teeth and may also lead to slightly more children being free of tooth decay. Finally, it concluded "Our confidence in the evidence is limited because this review included studies in which communities were deliberately selected to have changes to fluoride levels in the water supply."

We ask the Washington State Board of Health to continue to support this time-tested public health measure by adopting an optimal level of fluoride for communities that choose to augment their water supplies. We do not support the petition to recommend not adding chemicals, such as fluoride, to drinking water to treat or prevent disease in humans or animals.

Respectfully

Jim Sledge, DDS

Bob Lutz, MD MPH

From: bill teachingsmiles.com
Sent: 7/26/2025 10:23:29 AM
To: DOH WSBOH
Subject: August 20, 2025 WSBH Meeting Request

External Email

Dear Washington State Board of Health,

In preparation for the August 20, 2025, Board of Health meeting agenda, I am requesting 15 minutes of time to provide an "Observers Report" of the Fluoridation Pannel's evidence reviewed this year on fluoridation.

July 23, 2025, I was privileged to be invited to Washington DC, to provide expert evidence to the FDA CDER as hosted by the Reagan Udall Foundation. They even paid my expenses. The title of the full day program was, "Use of Orally Ingestible Unapproved Prescription Drug Products Containing Fluoride in the Pediatric Population." In other words, fluoride pills and drops usually prescribed where the water is not fluoridated.

My presentation to you, the Board of Health, will be an edited version of what I presented to the FDA CDER.

To approve a drug, the FDA CDER requires "substantial evidence of effectiveness," that always to my knowledge requires high quality randomized controlled trials, RCTs. RCTs are effectively required.

In other words, benefit (effectiveness) must be determined at a specific dosage and only then can safety be studied at that specific dosage. "The dose makes the poison" so we must know the dose that is effective so we can then determine whether at that dose fluoride is indeed safe.

The FDA CDER Director, George Tidmarsh MD PhD, Stanford, pediatric cancer specialist, former drug industry executive, highly qualified, was sitting under my nose in the front row, fully focused on the meeting. His response to the science was telling. He may make some mistakes in the future, but he understands the drug regulatory process and the importance of RCTs.

Thank you for my request for time to provide audio visual evidence for the Board's consideration on an unapproved drug mass medicated to many in Washington State.

Bill

Bill Osmunson DDS MPH
425.466.0100

From: Korndorfer, Jim H (DOH)
Sent: 8/6/2025 4:02:30 PM
To: DOH WSBOH,DOH IT Service Desk
Subject: Re: Petition for Early Access to DEXA Scans for At-Risk Young Women in WA



attachments\222D3A8FA2B443AC_image.png

This email looks legit because based on reading what it has to say and where it is coming from on the email address. WaTech is starting to send out the Phishing emails out again to test people's knowledge to see how much they know about Cybersecurity.

Here is some information we've been asked to provide by our security team that can assist you in this!

As for phishing emails! So as you're aware, Phishing emails are fraudulent messages designed to trick users into providing personal information or clicking on malicious links. These emails often appear to come from legitimate sources but contain subtle signs of deception. The Security team has asked us at the Service Desk not to be the judge if an email you receive is phishing or not, instead they want us collectively as an agency to develop an instinct for it.

How to report a suspicious email (visual instructions here):
https://stateofwa.sharepoint.com/sites/DOH-itservicedesk/SitePages/Identifying-Suspicious-Emails.aspx <https://stateofwa.sharepoint.com/sites/DOH-itservicedesk/SitePages/Identifying-Suspicious-Emails.aspx>

1. With the message open, click the Report Message button that is in your Outlook Ribbon and choose "Phishing" when prompted.
2. Choose "Don't show me this message again" and then click Report.
3. Delete the email - after reporting, delete the email from the inbox to prevent accidental interaction in the future

Thank you for the continued vigilance and cooperation in keeping DOH's digital environment secure!

Jim Korndorfer

He/Him

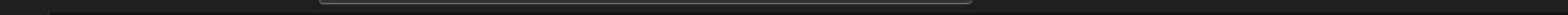
IT Support Technician 2

Health Tech – Office of Innovation & Technology

Washington State Department of Health

jim.korndorfer@doh.wa.gov

360-236-4357 | doh.wa.gov


Home
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New
Ignore
Block
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Report
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Chat
Share to Teams
Sweep
Move
Rules
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Categorize
Flag
Pin
Snooze
Policy
Print
More apps
Discover groups
Undo
New
Respond
Teams
Move
Quick steps
Tags
Print
Add-ins
Find
Undo

From: DOH WSBOH <WSBOH@SBOH.WA.GOV>
Sent: Monday, August 4, 2025 1:09 PM
To: DOH IT Service Desk <ITServiceDesk@doh.wa.gov>
Cc: Larson, Michelle L (SBOH) <Michelle.Larson@sboh.wa.gov>
Subject: FW: Petition for Early Access to DEXA Scans for At-Risk Young Women in WA

Hi DOH IT team.

We found this email in our junk folder and would like your help in verifying if it is a trusted email and if it is safe for us to click the link they have listed at the bottom of the email.

Thank you!

-----Original Message-----
From: Luca Hutton <luca-hutton@outlook.com>
Sent: Saturday, June 21, 2025 1:13 PM
To: DOH WSBOH <WSBOH@SBOH.WA.GOV>
Subject: Petition for Early Access to DEXA Scans for At-Risk Young Women in WA

External Email

Dear Members of the Washington State Board of Health, My name is Luca Hutton, and I am a student petitioning for expanded access to DEXA bone density scans for young women under age 65 who are at risk for early bone loss or osteoporosis.
Currently, DEXA scans are often only covered by insurance for individuals over 65. However, extensive medical research shows that bone loss can begin much earlier, especially for women affected by conditions such as:

* Eating disorders
* Low body weight or BMI
* Hormonal imbalances or amenorrhea
* Chronic illnesses or steroid use
* Family history of osteoporosis

One study found that even healthy women aged 20–36 showed early signs of osteoporosis or osteopenia. Yet without coverage or awareness, many young women remain undiagnosed until a fracture occurs. These fractures can be life-altering and preventable.
We ask that the Washington State Board of Health:

1. Advocate for earlier screening access and coverage for at-risk young populations.
2. Launch a state-level public awareness campaign about early bone loss.
3. Support low-cost or mobile DEXA screening programs in underserved areas.

Linked below is my completed petition for Adoption, Amendment, or Repeal of a State Administrative Rule.
Thank you for your leadership and your commitment to public health. I look forward to your support in improving access to essential osteoporosis screening and helping young women in Washington State.
Sincerely,
Luca Hutton
Luca-hutton@outlook.com <mailto:Luca-hutton@outlook.com> Dexa Scan Petition Form <file:///C:/Users/luca-/Downloads/DEXA%20Scan%20Petition.pdf>

From: bill teachingsmiles.com
Sent: 6/7/2025 10:33:51 AM
To: DOH WSBOH,Kwan-Gett, Tao (DOH)
Subject: The Dose Makes the Poison

attachments\E4BF73A768C948F8_The Dose Makes the Poison.pdf

attachments\559A6833D9B440EC_image.png

External Email

Dear Board of Health Members, (pdf format attached)

"The Dose Makes the Poison" has been repeated at the Board of Health.

The phrase and concept emphasize that toxicity is dependent on quantity—meaning even harmless substances can be harmful at high doses, while toxic substances can be safe at low doses. A first step in considering toxins; however, an oversimplification.

We are not all built the same. Individual Sensitivities – People react differently to substances due to genetic, health, or environmental factors. Some may be highly sensitive to small doses of a chemical, making the principle too generalized.

With fluoridation, over-generalization is a serious problem. Even if fluoridation were safe if everyone drank the same amount of water, some drink many times more than the average person. And women tend to drink significantly more water when pregnant.

Chronic vs Acute Dosage. An illustration that water can be a poison is valid. The average person drinks about 365 liters of water a year at one liter per day. Yet drinking 6 liters in three hours has caused death in a human.

However, chronic low-level exposure to fluoride can cause harm over time. For example, lead, asbestos, fluoride, tobacco, DDT and thousands of toxins may not be acutely poisonous in small doses but can accumulate and cause severe health issues over time with chronic low dose.

Age can also pose a significant variable. Not only due to individual size but also the developmental stage of the fetus, infant, or child.

Chemical Interactions – The phrase assumes a single substance in isolation, but chemicals can interact in unpredictable ways. A non-toxic dose of one substance may become harmful when combined with another.

Once again, the illustration of 300 mice is applicable. 100 mice were given toxin "A" increasing in dosage until the first mouse died, LD1. A second 100 mice were given toxin "B" until the first mouse died, LD1 for this group. The third 100 mice were given the LD1 of both toxin "A" and toxin "B." How many mice died? All 100.

Race and gender can also be factors. The graph below compares the blood lead levels in two groups, one in mostly non-fluoridated counties and the other in mostly fluoridated counties. Fluoridation increases the blood lead level of children, and it is greater in African-Americans than with Mexican heritage.

Nonlinear Effects – Some substances don’t follow a simple dose-response relationship. Hormones, endocrine disruptors, and certain pharmaceuticals can have disproportionately strong effects even at very low concentrations.

Irreversible Damage – Some toxins cause irreversible effects even at small doses, such as carcinogens or mutagens, which may trigger harmful biological changes at any level of exposure.

Safety Factor - An even greater safety factor must be used when authorities remove liberty and individual consent with fluoridation.

Sincerely,

Bill Osmunson DDS MPH

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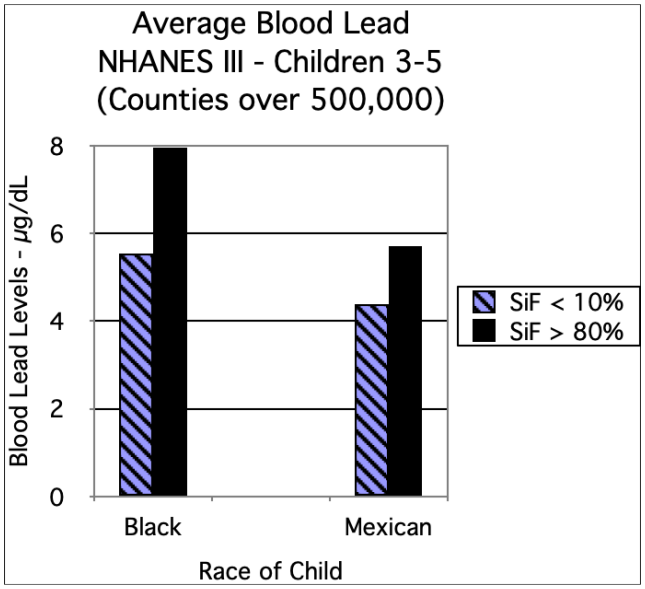
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Irreversible Damage – Some toxins cause irreversible effects even at small doses, such as carcinogens or mutagens, which may trigger harmful biological changes at any level of exposure.

Safety Factor - An even greater safety factor must be used when authorities remove liberty and individual consent with fluoridation.

Sincerely,

Bill Osmunson DDS MPH

_____

From: Bill Osmunson
Sent: 8/10/2025 2:12:39 PM
To: DOH WSBOH
Cc:
Subject: Public Comment August 2025 Board Meeting


attachments\B21888367F29465A_RCW43.20.050_Fluoridation_Legal_Letter.odt


attachments\E58177E3A240411E_RCW43.20.050_FDA_Gap_Analysis_Letter.docx

External Email

Dear Board of Health,

Please provide these public comments to the Board of Health for their review and consideration.

Bill

Bill Osmunson DDS MPH

Bill Osmunson, DDS, MPH
10300 181st Ave SE
Issaquah, WA 98027 425-466-
0100 bill@teachingsmiles.com

August 10, 2025

To:
Washington State Board of Health
PO Box 47990
Olympia, WA 98504-7990

2. Re: Legal and Ethical Duty Under RCW 43.20.050 to Assure Safe and Reliable Drinking Water

I am a retired dentist with a master's degree in public health. I have practiced dentistry for over 40 years, including 25 years supporting water fluoridation and 20 years examining the scientific and regulatory basis for its safety and efficacy. I write to you today as a concerned health professional and citizen, not as an attorney, to address what I believe is a significant conflict between the Board's statutory duty under **RCW 43.20.050** and its current rules authorizing and regulating the addition of fluoride to public water supplies.

1. The Board's Mandatory Duty

RCW 43.20.050(2)(a) states:

"The state board of health shall adopt rules controlling public water supplies, to assure safe and reliable public drinking water and to protect the public health."

This is an affirmative, non-discretionary duty. The Legislature has placed on the Board the responsibility to assure — not assume — the safety of drinking water provided to Washington residents.

3. The Permissive Fluoridation Law

Under RCW 57.08.012, a water district may choose to fluoridate, either by board action or through a public vote. This statute is permissive and does not require fluoridation. It also does not relieve the Board of its independent duty to ensure that any additive to drinking water is safe.

4. The Conflict

When the Board adopts rules under WAC 246-290-460 setting allowable fluoride concentrations for fluoridated systems, it is effectively authorizing a practice that has never been evaluated or approved under the U.S. Food and Drug Administration's (FDA) New Drug Application (NDA) process, despite meeting the statutory definition of a drug (21 U.S.C. § 321(g)(1)(B)) when added for the prevention of dental caries.

EPA's jurisdiction under the Safe Drinking Water Act is limited to controlling contaminants to avoid known or anticipated adverse effects — it does not evaluate therapeutic benefit, appropriate dosage for individuals, or long-term systemic safety in vulnerable populations. By deferring to EPA standards, the Board is not fulfilling its own statutory mandate to assure safety.

5. Absence of Proof of Safety

The 2024 Cochrane Review confirms there are no randomized controlled trials of water fluoridation demonstrating safety or significant efficacy. Multiple recent National Institutes of Health–funded studies

suggest potential neurodevelopmental risks at current exposure levels, particularly for infants. Without conclusive safety data, continued authorization of fluoridation conflicts directly with the Board's legal duty.

6. Legal and Ethical Implications

Under Washington's Administrative Procedure Act, a rule is arbitrary and capricious if it is adopted without consideration of all relevant factors or without a rational basis. A rule allowing fluoridation without proof of safety could be vulnerable to legal challenge. Ethically, public health policy requires affirmative demonstration of safety before mass exposure, not after harm is shown.

7. Requested Action

I respectfully request that the Board:

1. Acknowledge its non-discretionary duty under RCW 43.20.050 to assure the safety of drinking water,including all additives.
2. Suspend enforcement of WAC 246-290-460 allowing fluoridation until safety and efficacy aredemonstrated to FDA drug-approval standards.
3. Formally request that FDA CDER evaluate community water fluoridation under the NDA process, aswould be required for any other ingestible product intended for disease prevention.

I reiterate that I am not a lawyer, and this letter is not legal advice. It is my professional opinion, based on decades of clinical practice and public health study, that the Board's current approach to fluoridation is inconsistent with both the letter and the intent of RCW 43.20.050.

Respectfully submitted,

Bill Osmunson, DDS, MPH
Retired Dentist & Public Health Professional

Bill Osmunson, DDS, MPH

10300 181st Ave SE

Issaquah, WA 98027

425-466-0100

August 10, 2025

To:

Washington State Board of Health

PO Box 47990

Olympia, WA 98504-7990

Re: Gap Analysis and Legal Duty Under RCW 43.20.050 – Pathway to FDA CDER Review of Community Water Fluoridation

I am a retired dentist with a Master of Public Health, writing as a concerned health professional and on behalf of Washington Action for Safe Water—not as legal counsel—to highlight critical gaps in the oversight and safety assurance of community water fluoridation (CWF). This letter correlates Washington's statutory mandate under RCW 43.20.050(2)(a) with the preconditions required by the U.S. Food and Drug Administration's Center for Drug Evaluation and Research (FDA CDER) for systemic drug approval.

1. Legal Duty — RCW 43.20.050(2)(a)

Washington law mandates:
"The state board of health shall adopt rules controlling public water supplies, to assure safe and reliable public drinking water and to protect the public health."

This affirmative duty compels the Board to ensure safety before approving or supporting any intervention—including CWF.

2. FDA CDER Approval Pathway: NDA Gap Analysis

The table below compares core FDA NDA requirements to the current status of community water fluoridation.

FDA NDA Requirement	FDA's Expectation	Current Fluoridation Status
Chemistry & Manufacturing (CMC)	Precise identity, purity specifications, GMP documentation	Industrial-grade silicofluorides; NSF certification only; no FDA-reviewed CMC data
Preclinical Toxicology	Animal studies (acute, chronic, developmental, neurotoxicity) under GLP	No comprehensive modern GLP toxicology for systemic fluoride at 0.7 mg/L
Human Pharmacology / Safety	PK, metabolism, and safety in vulnerable groups	No formal PK or margin-of-safety data for infants, renal impairment, or genetic variations
Efficacy from RCTs	Adequate, well-controlled clinical trials demonstrating benefit	No RCTs; contemporary systematic reviews (e.g., Cochrane 2024) report low-certainty benefit evidence
Labeling Requirements	Approved indications, dosing, contraindications, warnings	No FDA-approved labeling; no individualized dosage control
Risk–Benefit Assessment	Balanced analysis across all populations and exposure ranges	Unresolved risks: dental fluorosis, possible neurodevelopmental effects; uncertain therapeutic margin

3. Regulatory Conflict and Board Obligations

The Board currently bases fluoridation rules (WAC 246-290-460) primarily on EPA contaminant standards and HHS/CDC recommendations. However, EPA evaluates risk of harm, not therapeutic benefit, dosage appropriateness, or long-term systemic safety. FDA is the only agency with authority to require proof of both safety and efficacy for substances intended to prevent disease. By endorsing fluoridation without resolving these NDA-level gaps, the Board risks falling short of its statutory duty to assure safety.

4. Recommended Path Forward

1) Commission a full gap analysis comparing CWF to FDA NDA requirements.
2) Suspend fluoridation rules until CWF safety and efficacy are demonstrated to FDA drug-approval standards.
3) Formally request that FDA CDER evaluate CWF via the NDA process.
4) Ensure transparent public communication on these unresolved gaps and intended corrective actions.

5. Closing

Your ruling authority carries the non-delegable duty to assure safe and reliable drinking water. Current policy permitting fluoridation, without addressing the FDA-level gaps noted above, risks both legal compliance and public trust. While I am not a lawyer, my decades of clinical and public health experience compel me to urge immediate action.

Respectfully submitted,

Bill Osmunson, DDS, MPH

Retired Dentist & Public Health Professional

_____

From: bill teachingsmiles.com
Sent: 6/22/2025 11:20:44 AM
To: DOH WSBOH
Cc:
Subject: Fw: Public comment: Limitations to the Principle: "The Dose Makes the Toxin"

attachments\330D0D0739DF4006_Dose Makes the Toxin.pdf

attachments\4C871452BBE74503_image.png

External Email

_____

From: bill teachingsmiles.com
Sent: Sunday, June 22, 2025 11:17 AM
To: Johnson, Laura W (DOH) <laura.johnson@doh.wa.gov>; Jenks, Lauren (DOH) <lauren.jenks@doh.wa.gov>; Kwan-Gett, Tao (DOH) <taosheng.kwan-gett@doh.wa.gov>
Subject: Public comment: Limitations to the Principle: "The Dose Makes the Toxin"

To: Fluoridation Panel Members and Board of Health Members,
From: Bill Osmunson, DDS, MPH]
Date: June 22, 2025 pdf attached
Subject: Limitations to the Principle: "The Dose Makes the Toxin"

I. Introduction

The principle "the dose makes the toxin", a long-standing concept in toxicology, holds that a substance only becomes harmful at a certain dose. While foundational, this maxim is increasingly inadequate when evaluating substances like fluoride, particularly in the context of community water fluoridation. Current scientific and ethical standards call for a more nuanced approach to risk assessment and public health policy.

II. Why the Dose Principle Breaks Down for Fluoride

1. Nonlinear and Low-Dose Effects

Fluoride shows biological effects at doses once presumed safe. Studies funded by NIH and Health Canada link low prenatal fluoride exposure to reduced IQ in children. These findings reveal non-monotonic dose-responses inconsistent with traditional toxicological assumptions.

For example, the data graphed by Dr. Thiessen of Iida & Kumar caries and dental fluorosis illustrates a possible decline in caries and then as the dose increases the caries increases, a possible non-linear effect of fluoride.

2. Vulnerable Populations

A uniform concentration of fluoride results in varying doses across individuals. Infants, those with kidney impairment, developing fetuses, and infants on formula made with fluoridated water are especially vulnerable to adverse effects. No single "optimal" dose can ensure safety for all.

3. Chronic and Cumulative Exposure

Fluoride toxicity can be acute but more common is chronic. Fluoride accumulates in bones and soft tissue over time. Multiple sources—water, food, dental products—contribute to a total burden that exceeds safe levels for many.

4. Co-Exposures and Chemical Mixtures

Fluoride is rarely encountered alone. Water fluoridation introduces additional contaminants such as arsenic and lead, which may act synergistically. Evaluating fluoride

in isolation does not reflect real-world exposures.

5. Timing of Exposure

The timing of fluoride exposure is critical. Prenatal and early-life exposures, even at low doses, can impact brain development and thyroid function. Effects seen in utero or during infancy may not occur later in life at the same dose.

6. Ethical and Regulatory Challenges

Fluoride meets the legal definition of a drug under Washington State law (RCW 69.38.010). Yet water fluoridation delivers a pharmacologically active substance without prescription, dosage control, or informed consent. This contravenes ethical standards for public health interventions.

III. Conclusion

"The dose makes the toxin" is no longer a sufficient basis for evaluating fluoride safety. Modern evidence shows harm at low, chronic doses, particularly among sensitive populations. Public health policy must reflect current science and ethical obligations, including protection of the most vulnerable and respect for individual autonomy.

Bill Osmunson, DDS, MPH
Email: bill@teachingsmiles.com
Phone: 425.466.0100

To: Fluoridation Panel Members and Board of Health Members,

From: Bill Osmunson, DDS, MPH]
Date: June 22, 2025

Subject: Limitations to the Principle: “The Dose Makes the Toxin”

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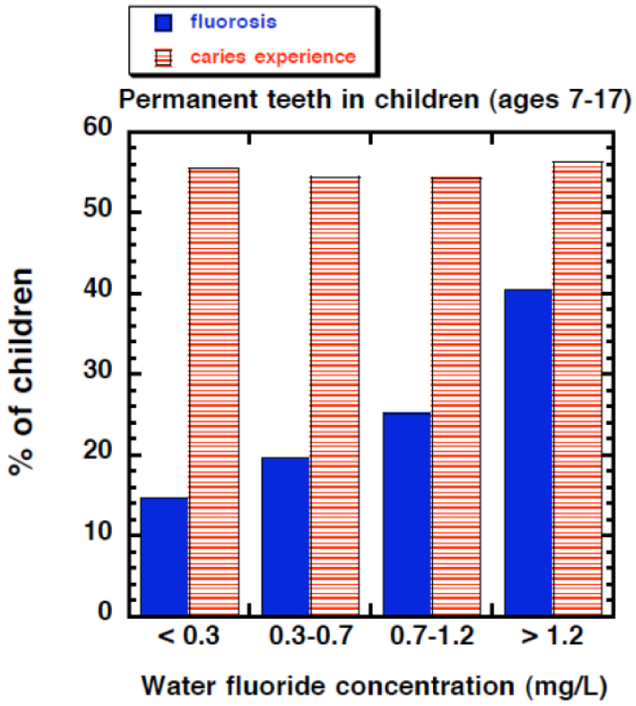
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Iida, H., and Kumar, J.V. 2009. The association between enamel fluorosis and dental caries in U.S. schoolchildren. JADA 140:855-862.



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Bill Osmunson, DDS, MPH
Email: bill@teachingsmiles.com
Phone: 425.466.0100

_____

From: Crystal McGaffin
Sent: 8/14/2025 11:40:19 AM
To: DOH WSBOH
Cc:
Subject: WSDA supports Water Fluoridation

External Email

Hello,

On behalf of the Washington State Dental Association (WSDA) and the over 4,000 member dentists, I am writing to express our continued support for community water fluoridation as a safe, effective, and equitable public health measure.

The WSDA strongly believes that opening any rulemaking that could weaken Washington's fluoridation policies including changes to the maximum contaminant levels or alterations to public messaging will have unintended consequences for local programs. While we appreciate the Board's ongoing commitment to public health, we want to express our concerns that opening state level rulemaking on such a topic, particularly when considering that the EPA is expected to address fluoride regulations in the near future, would create unnecessary confusion and duplicate regulatory processes while increasing the risk of eroding decades of proven oral health benefits for our state residents.

As recent state and federal dental Medicaid reductions take effect, Washington will face intersecting public health pressures as other critical resources become increasingly scarce. Without strong prevention measures, the burden of these setbacks with fall hardest on families and seniors who can least afford it. Upholding community water fluoridation remains the most effective and cost-efficient strategy to protect all residents, regardless of age, income, or insurance status.

The WSDA respectfully asks the Board to maintain its current support for these practices. Thank you for your consideration and allowing the WSDA the opportunity to be involved in these discussions.

Best,

Crystal McGaffin
Dental Benefits Advocacy Manager
crystalm@wsda.org <mailto:crystalm@wsda.org> | (206)-973-5231

Washington State Dental Association | wsda.org
<https://gcc02.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.wsda.org%2F&data=05%7C0

Book time with Crystal McGaffin

<https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Foutlook.office.com%2Fbookwithm

Dennis Worsham
Secretary of Health, Department of Health

A lifelong Washingtonian raised in Eastern Washington, Dennis brings 32 years of distinguished service in Washington State's public health system to this critical role. His career began at the Snohomish Health District in the 1990s, where he discovered his passion for public health's mission and addressing systemic health inequities while working as health educator during the HIV/AIDS epidemic. Most recently, he served as the first Director of the Snohomish County Health Department since January 2023. Dennis also brings a deep commitment to working collaboratively with all parts of the public health system including our Tribal, state, local, community and healthcare partners.

His extensive background includes:

- **Prior Executive Leadership Experience:** Interim Director of Public Health – Seattle & King County, and Deputy Secretary for Health at the Washington State Department of Health (2014-2016)
- **Diverse Public Health Expertise:** From TB control and HIV/STD/Hepatitis programs to chronic disease and other policy development, community partnerships, and communications
- **Deep Commitment to Partnerships:** Active involvement with the Washington State Public Health Association, Public Health Roundtable, Washington State Association of Public Health Officials, and Foundational Public Health Services committee
- **Lifelong Equity Champion**: A commitment to lead public health policy and practice through an equity and community-centered lens. He helped create the very first leadership positions in various agencies to advance organizational equity and health justice

Secretary Worsham's unique perspective, shaped by firsthand experience in both rural communities and large urban systems, positions him as a leader who understands that public health affects all of us — regardless of where we live or who we are. His vision centers on the understanding that public health challenges don't recognize geographic boundaries, and neither should our solutions.

“As Washington’s Secretary of Health, I am honored to serve and deeply committed to upholding and strengthening a public health system that is not only resilient, but also more just. It is critical that we continue to build a system that earns the trust of communities and strives for every person to have equitable access to the care and resources they need to thrive,” Worsham said. “Public health is not just about addressing illness and injury — it’s about preventing them. It’s about preparing for the unexpected, addressing root causes of health inequities, and investing in systems that protect people before a crisis begins. We must ensure health and wellbeing is not a privilege but a shared foundation for all.”

Yen Baynes
Community Engagement Lead

Yen Baynes (she/elle/ella) joined the Governor's Interagency Council on Health Disparities on July 1, 2025. She will lead community and Tribal engagement and outreach efforts, helping the Council build relationships with various partners and communities.

Yen brings over 20 years of experience in grassroots organizing, birth equity, disability justice, emergency preparedness, and CLAS (Culturally and Linguistically Appropriate Services), along with five years in Washington State government. Most recently, Yen served as a strategist at the Washington State Department of Health, supporting the integration of racial equity, trauma-informed practice, and community voice into statewide public health efforts.

Yen holds a Master's degree in Education with a focus on policy and community engagement. As a parent, advocate, and someone with lived experience navigating systems that were not built for all of us, she's committed to making equity work real, durable, and community-led. She steps into this role at a time when public health infrastructure is being actively dismantled for too many communities. She stands ready to share her expertise as we work collectively to provide the clarity, collaboration, and courage this moment demands.

From: bill teachingsmiles.com
To: AGOConsumer@atg.wa.gov
Cc: DOH WSBOH
Subject: Formal Complaint
Date: Sunday, June 22, 2025 9:05:43 AM

External Email

Formal Complaint to the Washington State Attorney General

Bill Osmunson, DDS, MPH
Email: bill@teachingsmiles.com

Issaquah, Washington
Phone: (425) 466-0100

Date: June 22, 2025

To:
Attorney General Bob Ferguson
Office of the Attorney General
1125 Washington St SE
PO Box 40100
Olympia, WA 98504-0100
Email: AGOConsumer@atg.wa.gov

Subject: Formal Complaint – Unlawful Medicalization of Public Drinking Water Through Fluoridation

Dear Attorney General Ferguson,

I write to formally request an investigation and legal review of water fluoridation practices in Washington State, which appear to violate multiple provisions of state law and fundamental ethical principles.

Specifically:

1. **RCW 69.38.010** defines fluoride, when used to prevent disease, as a **legend drug**, requiring a prescription and professional oversight.

2. The Washington State Board of Pharmacy determined fluoride to be a legend drug in 2019.

3. Despite this, fluoride is being added to public water systems—**without prescriptions, informed consent, or medical supervision**—amounting to unauthorized mass drug

administration.

4. **RCW 57.08.012** permits fluoridation by public water districts, but **does not override the requirements of legend drug laws** or provide medical authority to water districts or the Department of Health.

5. **RCW 43.20.050(2)(a)** requires the Board of Health to adopt rules that **assure the safety of drinking water**. However, mounting scientific evidence—including the 2023 National Toxicology Program (NTP) review and federal court findings in *Food & Water Watch v. EPA*—suggest that fluoridated water may **pose a neurodevelopmental risk to fetuses and children**.

Numerous peer-reviewed studies, including Bashash et al. (2017), Green et al. (2019), and Till et al. (2020), link fluoride exposure during pregnancy to measurable IQ loss and neurodevelopmental harm. These findings have been acknowledged by a federal court as credible and serious.

Accordingly, I respectfully request:

- A formal opinion on whether the addition of fluoride to public drinking water without prescription or consent violates RCW 69.38.010;

- A review of whether the Department of Health or local water districts possess legal authority to bypass legend drug regulations;

- Immediate action to halt water fluoridation pending a full legal and scientific safety review;

- A directive ensuring that public health decisions comply with both Washington State law and the public's right to medical autonomy and safe drinking water.

This complaint is filed in the interest of protecting vulnerable populations—especially infants, children, pregnant women, and individuals with kidney disease or other fluoride sensitivities.

I would appreciate an acknowledgment of this complaint and notification of any actions initiated.

Sincerely,
Bill Osmunson, DDS, MPH
bill@teachingsmiles.com
(425) 466-0100

RULE-MAKING ORDER
EMERGENCY RULE ONLY

CR-103E (December 2017)
(Implements RCW 34.05.350 and 34.05.360)

CODE REVISER USE ONLY

OFFICE OF THE CODE REVISER
STATE OF WASHINGTON
FILED

DATE: June 06, 2025
TIME: 7:26 AM

WSR 25-13-014

Agency: Department of Health

Effective date of rule:

Emergency Rules

☒ Immediately upon filing.

☐ Later (specify)

Any other findings required by other provisions of law as precondition to adoption or effectiveness of rule?

☐ Yes ☒ No If Yes, explain:

Purpose: WAC 246-282-006, Washington state Vibrio parahaemolyticus control plan. Vibrio parahaemolyticus (Vp) is a naturally occurring bacteria found in marine waters. Molluscan bivalve shellfish acquire Vp through filter feeding. Humans who consume raw or under-cooked shellfish containing Vp can develop an intestinal disease called vibriosis.

Chapter 246-282 WAC establishes the minimum performance standards for growing, harvesting, processing, packing, storage, transporting, and selling of shellfish for human consumption. These rules do not apply to persons who conduct activities limited to retail food service, personal use, and transporting as a common carrier of freight.

WAC 246-282-006 establishes the control plan for the months of May 1st through September 30th and are an extension of the NSSP Model Ordinance (U.S. Food and Drug Administration National Shellfish Sanitation Program, Guide for the Control of Molluscan Shellfish). The control plan requires harvesters to comply with time to cooling requirements which are set to be more stringent from July 1 through September 30 because of the increased risk of vibriosis during these times due to warm water and low tide conditions.

Due to current early season high temperatures this year, coinciding with midday low tides, this emergency rulemaking is necessary to protect public health by modifying the existing strictest harvest control requirements, which currently start July 1st, by modifying the start date for the strictest harvest control requirements from July 1 to June 6.

The State Board of Health (Board) is currently engaged in permanent rulemaking on this topic and filed a Preproposal Statement of Inquiry (CR-101) on February 23, 2022, under WSR 22-06-034, to consider revisions to the chapter to modify harvest control requirements by setting more proactive measures to prevent illnesses and protect public health. The rule revision may also include updating definitions, seed size, and other technical and editorial changes as needed.

Beginning in March 2022 and until permanent rulemaking can be completed, the Board has delegated emergency rulemaking authority to the Department of Health if heat-wave conditions occur prior to July 1st.

Citation of rules affected by this order:

New: None
Repealed: None
Amended: WAC 246-282-006
Suspended: None

Statutory authority for adoption: RCW 69.30.030 and 43.20.050

Other authority: None

EMERGENCY RULE

Under RCW 34.05.350 the agency for good cause finds:

☒ That immediate adoption, amendment, or repeal of a rule is necessary for the preservation of the public health, safety, or general welfare, and that observing the time requirements of notice and opportunity to comment upon adoption of a permanent rule would be contrary to the public interest.

☐ That state or federal law or federal rule or a federal deadline for state receipt of federal funds requires immediate adoption of a rule.

Reasons for this finding: To reduce the threat to public health, amending the time of harvest to cooling requirements to a more protective control season immediately is necessary. The current Vp Control Plan, last updated in 2015, establishes a control season between May 1 and September 30. However, the strictest time-to-cooling requirements for oysters currently begin July 1. Due to forecast early season high temperatures and midday low tides in June 2025, this emergency rule shifts the start of these stricter harvest controls to June 6, 2025, to reduce the risk of vibriosis.

From June 26 to July 2, 2021, the National Weather Service in Seattle reported a long-duration, unprecedented heat wave throughout the Pacific Northwest. Shellfish-related Vp illnesses increased sharply. This prompted the Board to initiate permanent rulemaking and demonstrated the need for more flexible rules. Heat wave conditions occurred in May 2023, and emergency rules were adopted. Due to early season temperatures forecast to occur in June 2025, coinciding with midday low tides, this emergency rule is necessary to protect public health.

Note: If any category is left blank, it will be calculated as zero. No descriptive text.

Count by whole WAC sections only, from the WAC number through the history note. A section may be counted in more than one category.

The number of sections adopted in order to comply with:

Federal statute:	New 0	Amended 0	Repealed 0
Federal rules or standards:	New 0	Amended 0	Repealed 0
Recently enacted state statutes:	New 0	Amended 0	Repealed

The number of sections adopted at the request of a nongovernmental entity:

New 0 Amended 0 Repealed 0

The number of sections adopted on the agency's own initiative:

New 0 Amended 1 Repealed 0

The number of sections adopted in order to clarify, streamline, or reform agency procedures:

New 0 Amended 0 Repealed 0

The number of sections adopted using:

Negotiated rule making:	New 0	Amended 0	Repealed 0
Pilot rule making:	New 0	Amended 0	Repealed 0
Other alternative rule making:	New 0	Amended 1	Repealed 0

Date Adopted: 6/6/2025	**Signature:** 
Name: Kristin Peterson, JD, for Jessica Todorovich, MS	
Title: Chief of Policy for Acting Secretary of Health	

AMENDATORY SECTION (Amending WSR 15-08-083, filed 3/31/15, effective 5/1/15)

WAC 246-282-006 Washington state *Vibrio parahaemolyticus* control plan. (1) This section establishes the Washington state *Vibrio parahaemolyticus* control plan (control plan) for the months of May 1st through September 30th (control months). The requirements of this section are an extension of the NSSP Model Ordinance.

(2) All harvesters and shellfish dealers harvesting or delivering oysters to a certified shucker packer for shucking or postharvest processing (PHP) during the control months must label the oysters with a harvest tag stating "For shucking by a certified dealer" or "For PHP by a certified dealer." Oysters harvested and tagged in compliance with this subsection are exempt from subsections (3) through (20) of this section.

(3) The following definitions apply throughout this section:

(a) "Single-source *Vibrio parahaemolyticus* case" or "case" means a laboratory-confirmed *Vibrio parahaemolyticus*-associated illness or illnesses with a common exposure that are reported to the department. The case must:

(i) Be associated with commercially harvested shellstock;

(ii) Not involve documented postharvest abuse; and

(iii) Be traced back to a single growing area.

(b) "Control months" means May 1st through September 30th.

(c) "Cool" or "cooling" means to:

(i) Adequately ice or place in a controlled environment with a temperature of 45°F (7.2°C) or less; and

(ii) Reach and maintain an internal oyster tissue temperature of 50°F (10°C) or less.

(d) "Harvest temperature" means the water temperature or internal oyster tissue temperature at the time of harvest. The harvester or shellfish dealer shall state whether they use water temperature or internal oyster tissue temperature for harvest temperature in their harvest plan.

(4) All harvesters and shellfish dealers harvesting oysters during the control months shall report the volume of oysters harvested. This information must be reported by month, oyster species, size class, and growing area for all control months. This information must be reported by December 31st each year. Harvesters and shellfish dealers that do not submit this information to the department may not harvest oysters during the control months during the next calendar year.

(5) Harvesters and shellfish dealers harvesting oysters during the control months shall complete, submit to the department, and keep on file a current *Vibrio parahaemolyticus* harvest plan. In order for the department to review the harvest plan prior to May 1st, the harvest plan must be submitted by March 1st each year unless no changes have been made to the existing harvest plan. Harvesters and shellfish dealers shall sign and date their harvest plan each year and make it available to the department upon request.

(6) The harvest plan must:

(a) Describe the harvest, temperature collection, cooling, and conveyance methods.

(b) Include an example of the harvest temperature record designed to meet the requirements in subsection (11) of this section.

(c) Identify if water temperature or internal oyster tissue temperature is used to meet the requirements in subsection (11) of this section and specifically how this measurement will be taken.

(7) The department shall review and either approve or deny the harvest plan within thirty days of receipt. If the department denies approval of the harvest plan, the department shall notify the applicant of the decision in writing stating the reasons for the denial and providing the opportunity to correct the deficiencies. Harvesters and shellfish dealers may not harvest oysters during the control months unless the department has approved the plan.

(8) Time of harvest to cooling requirements and harvest controls are based on a risk categorization of each growing area. The department shall assign each growing area a category of 1, 2, or 3 (where 1 corresponds to the least stringent and 3 the most stringent controls) based on the number of cases that occurred during the previous consecutive five-year period within the control months and were attributed to that growing area.

(9) The department shall categorize coastal growing areas in Willapa Bay and Grays Harbor as Category 1 for the first year of implementation attributing no illnesses to these areas for the years 2010 to 2014. For subsequent years, the department shall categorize coastal growing areas based on the criteria in subsection (8) of this section.

(10) The department shall complete risk categorization and publish a list of all growing areas by risk category no later than February 1st annually. The department shall use a rolling five-year average number of cases to calculate risk categories as follows:

(a) Category 1: An average of 0.2 or fewer cases attributed to the growing area over a five-year period.

(b) Category 2: An average of more than 0.2, but less than 1.0 cases attributed to the growing area over a five-year period.

(c) Category 3: An average of 1.0 or more cases attributed to the growing area over a five-year period.

(11) Time of harvest begins after the first oysters to be harvested are exposed to the air. Time of harvest to cooling requirements and harvest controls are as follows:

(a) Category 1:

Requirements:	**Time to Cooling:**
Except as noted below, the time of harvest to cooling requirement from ((~~June~~)) May 1st through September 30th is:	9 hours
When ambient air temperature at harvest is greater than 90°F, the time of harvest to cooling requirement is:	7 hours
When harvest temperature is between 68°F and 70°F from ((~~July 1st through August 31st~~)) June 6th through September 30th, the time of harvest to cooling requirement is:	5 hours
Harvest Control: From ((~~July 1st through August 31st~~)) June 6th through September 30th, harvest is not allowed for twenty-four hours when harvest temperature is above 70°F.	

(b) Category 2:

Requirements:	Time to Cooling:
Except as noted below, the time of harvest to cooling requirement from May 1st through September 30th is:	7 hours
When ambient air temperature at harvest is greater than 85°F, the time of harvest to cooling requirement is:	5 hours
When harvest temperature is between 66°F and 68°F from ((~~July 1st through August 31st~~)) June 6th through September 30th, the time of harvest to cooling requirement is:	3 hours
Harvest Control: From ((~~July 1st through August 31st~~)) June 6th through September 30th, harvest is not allowed for twenty-four hours when harvest temperature is above 68°F.	

(c) Category 3:

Requirements:	Time to Cooling:
Except as noted below, time of harvest to cooling requirement from May 1st through September 30th is:	5 hours
When ambient air temperature at harvest is greater than 80°F, the time of harvest to cooling requirement is:	3 hours
When harvest temperature is between 64°F and 66°F from ((~~July 1st through August 31st~~)) June 6th through September 30th, the time of harvest to cooling requirement is:	1 hour
Harvest Control: From ((~~July 1st through August 31st~~)) June 6th through September 30th, harvest is not allowed for twenty-four hours when harvest temperature is above 66°F.	

(d) When a harvester or shellfish dealer places oysters in a container or conveyance, but does not remove them from the tide flat as part of their harvest and the harvest exceeds the time to cooling requirements in subsection (11) of this section, then the oysters in the container or conveyance must be covered by the tide for a minimum of four hours before harvest can be completed.

(12) Harvesters and shellfish dealers shall take the following measurements at the times specified below and record this information in a harvest temperature record for each harvest site for all harvests occurring within the control months. Harvesters and shellfish dealers shall take these measurements with a thermometer that is verified weekly using manufacturer specifications or with a method approved in a harvest plan. Thermometer verification must be documented and maintained with operational records. Harvesters and shellfish dealers shall record the following measurements and the date and time they were taken in the record, maintain the record for three years, and make the record available to the department upon request:

(a) Air temperature at time and location of harvest; and

(b) Harvest temperature at time and location of harvest. Harvesters and shellfish dealers using water temperature for harvest temperature shall take water temperature at depth of oysters unless another method is documented in their harvest plan.

(13) Harvesters and shellfish dealers shall initiate cooling as soon as practical from the time of harvest and within the time of harvest to cooling requirements for the growing area where the oysters were harvested to ensure that the maximum number of hours is not exceeded.

(14) If the required time of harvest to cooling requirements are not met after removal from the tide flat, the harvester or shellfish dealer shall dispose of the oysters using one of the methods below and record the disposition on the harvest record:

(a) Destroy the oysters;

(b) Place the oysters within the original growing area or another approved growing area and allow a minimum of fourteen days before reharvesting; or

(c) Deliver the oysters to a certified shucker packer for shucking or PHP and attach a harvest tag meeting the requirements in subsection (2) of this section.

(15) If ownership of oysters is transferred prior to the oysters being cooled in accordance with the time of harvest to cooling requirements, the harvester shall include in the harvest record required under WAC 246-282-080 the:

(a) Temperatures recorded under subsection (12) of this section;

(b) Date, time, and person or entity to whom the oysters were transferred; and

(c) Growing area risk category for the harvested product.

(d) The receiving shellfish dealer shall meet the time of harvest to cooling requirements for the original harvest time.

(16) *Vibrio parahaemolyticus* training requirements are as follows:

(a) Harvesters and shellfish dealers shall complete an initial department-approved training specific to the requirements of this section prior to harvesting or shipping oysters during the control months.

(b) Harvesters and shellfish dealers shall complete department-approved refresher training within one year following any revision of this rule considered significant under RCW 34.05.328 or at least every five years.

(c) Those responsible for the on-site management of harvest activities must be trained by either:

(i) Harvesters and shellfish dealers at their operation who completed the department-approved training; or

(ii) The department.

(d) Harvesters and shellfish dealers shall record those trained in their operational records.

(17) A harvester or shellfish dealer may request a waiver from specific requirements of this section. The request must:

(a) Be in writing;

(b) Identify the requirement requested to be waived;

(c) State the reason for the waiver; and

(d) Provide supporting information.

(18) The department may grant a waiver request if it:

(a) Is consistent with the applicable standards and the intent of this section; and

(b) Provides a comparable level of public health protection to the requirement being waived.

(19) If the department approves a waiver request, the department shall notify the requestor of the decision in writing.

(20) If the department denies a waiver request, the department shall notify the requestor of the decision in writing stating the reasons for the denial. The requestor shall comply with the provision that was the subject of the waiver request.

(21) The department shall review this section to evaluate the effectiveness of the rules and determine areas where revisions may be necessary by November 2017.

CODE REVISER USE ONLY

RULE-MAKING ORDER
EMERGENCY RULE ONLY

CR-103E (December 2017)
(Implements RCW 34.05.350 and 34.05.360)

OFFICE OF THE CODE REVISER
STATE OF WASHINGTON
FILED

DATE: June 18, 2025
TIME: 8:30 AM

WSR 25-13-104

Agency: State Board of Health

Effective date of rule:

Emergency Rules

☒ Immediately upon filing.

☐ Later (specify)

Any other findings required by other provisions of law as precondition to adoption or effectiveness of rule?

☐ Yes ☒ No If Yes, explain:

Purpose: Testing of drinking water contaminants - State action levels (SALs) and state maximum contaminant levels (MCLs) in WAC 246-290-315.

The State Board of Health (board) has authority under RCW 43.20.050 to adopt rules for group A public water systems that are necessary to assure safe and reliable public drinking water and to protect the public health. Chapter 246-290 WAC, Group A Public Water Supplies, establishes standards and requirements for these water systems. The Department of Health (department) administers the rules.

To ensure safe drinking water, water must be tested for contaminants. The board establishes SALs and MCLs to ensure contaminate levels are below a certain threshold. The board sets criteria for the adoption of SALs and MCLs in WAC 246-290-315, and includes criteria that would apply upon federal adoption of MCLs. WAC 246-290-315(8) states that upon federal adoption of a MCL, the MCL will supersede a less stringent SAL and associated requirements, including monitoring and public notice.

The EPA published new federal standards for per- and polyfluoroalkyl substances (PFAS) on April 10, 2024, with an adoption date of June 25, 2024. These new standards include MCLs. This affects the board's rule and triggers the provision in WAC 246-290-315(8). The federal standards, however, have delayed effective dates for criteria and public health protections that are currently in place for Washington. According to the Washington state rules associated with the SALs, public water systems must notify customers of detections of PFAS above the SAL within 30 days of that detection. This is necessary to allow people the opportunity to protect themselves by using bottled water, securing a filter, or taking other measures. 30-day public notification is not effective for MCLs in the federal standard until April 2029. Without this amendment to WAC 246-290-315, customers served by group A public water systems will no longer be notified of dangerous levels of PFAS in their drinking water, which is a significant reduction in protections.

The board adopted an emergency rule on June 12, 2024, to amend WAC 246-290-315 such that the criteria would apply on the effective date of an MCL as set in the federal standard, not the adoption date, in order to maintain vital public health protections for drinking water safety. Along with the emergency rulemaking, the board initiated a permanent rulemaking to amend the rule language to align with the emergency provision and explore other protections. The CR-101, Preproposal Statement of Inquiry, for the permanent rulemaking was filed as WSR 24-20-093 on September 30, 2024. This 4th emergency rule continues the emergency rule originally filed on June 24, 2024 as WSR 24-14-016 and extended on October 22, 2024 and February 19, 2025 as WSR 24-21-138 and WSR 25-05-095, respectively, without change.

Citation of rules affected by this order:
New: None
Repealed: None
Amended: WAC 246-290-315
Suspended: None

Statutory authority for adoption: RCW 43.20.050(2)(a)

Other authority: None

EMERGENCY RULE

Under RCW 34.05.350 the agency for good cause finds:

☒ That immediate adoption, amendment, or repeal of a rule is necessary for the preservation of the public health, safety, or general welfare, and that observing the time requirements of notice and opportunity to comment upon adoption of a permanent rule would be contrary to the public interest.

☐ That state or federal law or federal rule or a federal deadline for state receipt of federal funds requires immediate adoption of a rule.

Reasons for this finding: The federal adoption date of the standards was June 25, 2024, at which point the MCLs and relative protections would have superseded the SALs. Because of the delayed effective date, currently active public health protections would have ended on that date. The Board finds that emergency adoption of this rule is necessary to preserve public health.

Note: If any category is left blank, it will be calculated as zero. No descriptive text.

Count by whole WAC sections only, from the WAC number through the history note. A section may be counted in more than one category.

The number of sections adopted in order to comply with:

Federal statute:	New 0	Amended 0	Repealed 0
Federal rules or standards:	New 0	Amended 0	Repealed 0
Recently enacted state statutes:	New 0	Amended 0	Repealed 0

The number of sections adopted at the request of a nongovernmental entity:

New 0 Amended 0 Repealed 0

The number of sections adopted on the agency's own initiative:

New 0 Amended 1 Repealed 0

The number of sections adopted in order to clarify, streamline, or reform agency procedures:

New 0 Amended 0 Repealed 0

The number of sections adopted using:

Negotiated rule making:	New 0	Amended 0	Repealed 0
Pilot rule making:	New 0	Amended 0	Repealed 0
Other alternative rule making:	New 0	Amended 1	Repealed 0

Date Adopted: June 18, 2025	**Signature:** 
Name: Michelle Davis, MPA	
Title: Executive Director, Washington State Board of Health	

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-315 State action levels (SALs) and state maximum contaminant levels (MCLs). (1) The department shall consider the following criteria to select a contaminant for developing a SAL:

(a) Drinking water contributes to human exposure to the contaminant.

(b) The contaminant is known or likely to occur in public water systems at levels of public health concern. Sources of occurrence information include, but are not limited to:

(i) Washington state department of agriculture;

(ii) Washington state department of ecology; and

(iii) Monitoring results reported in accordance with 40 C.F.R. 141.35.

(c) The contaminant has a possible adverse effect on the health of persons exposed based on peer-reviewed scientific literature or government publications, such as:

(i) An EPA health assessment such as an Integrated Risk Information System assessment;

(ii) Agency for Toxic Substances and Disease Registry toxicological profiles;

(iii) State government science assessment; and

(iv) EPA guidelines for exposure assessment such as the EPA exposure factors handbook.

(d) A certified drinking water lab can accurately and precisely measure the concentration of the contaminant in drinking water at and below the level of public health concern using EPA-approved analytical methods.

(2) After consideration of the criteria in subsection (1) of this section, the department may develop a SAL based on the following:

(a) Evaluation of available peer-reviewed scientific literature and government publications on fate, transport, exposure, toxicity and health impacts of the contaminant and relevant metabolites;

(b) An assessment based on the most sensitive adverse effect deemed relevant to humans and considering susceptibility and unique exposures of the most sensitive subgroup such as pregnant women, fetuses, young children, or overburdened and underserved communities; and

(c) Technical limitations to achieving the SAL such as insufficient analytical detection limit achievable at certified drinking water laboratories.

(3) The state board of health shall consider the department's findings under subsections (1) and (2) of this section when considering adopting a SAL under this chapter.

(4) Contaminants with a SAL.

(a) If a SAL under Table 9 of this section is exceeded, the purveyor shall take follow-up action as required under WAC 246-290-320. For contaminants where the SAL exceedance is determined based upon an RAA, the RAA will be calculated consistent with other organic contaminants per WAC 246-290-320(6) or other inorganic contaminants per WAC 246-290-320(3).

TABLE 9
STATE ACTION LEVELS

Contaminant or Group of Contaminants	SAL	SAL Exceedance Based On:
Per- and polyfluoroalkyl substances (PFAS)		
PFOA	10 ng/L	Confirmed detection
PFOS	15 ng/L	Confirmed detection
PFHxS	65 ng/L	Confirmed detection
PFNA	9 ng/L	Confirmed detection
PFBS	345 ng/L	Confirmed detection

(b) If a system fails to collect and submit a confirmation sample to a certified lab within ten business days of notification of the sample results, or as required by the department, the results of the original sample will be used to determine compliance with the SAL.

(5) The department shall consider the following when developing a state MCL:

(a) The criteria in subsection (1) of this section;

(b) Whether regulating the contaminant presents a meaningful opportunity to reduce exposures of public health concern for persons served by public water systems;

(c) The need for an enforceable limit to achieve uniform public health protection in Group A public water systems; and

(d) The need for an enforceable limit to support source water investigation and clean-up of a contaminant in drinking water supplies by responsible parties.

(6) In addition to the requirements in subsection (5) of this section, the department shall:

(a) Meet the requirements of subsection (2) of this section;

(b) Comply with the requirements in RCW 70A.130.010 to establish standards for chemical contaminants in drinking water;

(c) Consider the best available treatment technologies and affordability taking into consideration the costs to small water systems; and

(d) Determine that the probable benefits of the rule are greater than its probable costs, taking into account both the qualitative and quantitative benefits and costs.

(7) The state board of health shall consider the department's findings under subsections (5) and (6) of this section and follow the requirements under chapters 34.05 and 19.85 RCW when adopting a state MCL under this chapter.

(8) ((~~Upon federal adoption of an MCL~~)) When a federal MCL takes effect, the federal MCL will supersede a SAL or a less stringent state MCL, and the associated requirements, including for monitoring and public notice. If the federally adopted MCL is less stringent than a SAL or state MCL, the board may take one of the following actions:

(a) Adopt the federal MCL; or

(b) Adopt a state MCL, at least as stringent as the federal MCL, using the process in subsections (6) and (7) of this section.

RULE-MAKING ORDER
PERMANENT RULE ONLY

CR-103P (December 2017)
(Implements RCW 34.05.360)

CODE REVISER USE ONLY

OFFICE OF THE CODE REVISER
STATE OF WASHINGTON
FILED

DATE: June 17, 2025
TIME: 10:15 AM

WSR 25-13-093

Agency: Department of Health

Effective date of rule:

Permanent Rules

☒ 31 days after filing.

☐ Other (specify) _____ **(If less than 31 days after filing, a specific finding under RCW 34.05.380(3) is required and should be stated below)**

Any other findings required by other provisions of law as precondition to adoption or effectiveness of rule?

☐ Yes ☒ No If Yes, explain:

Purpose: Per- and polyfluoroalkyl substances (PFAS) – Adopting the National Primary Drinking Water Regulations (40 CFR Part 141) by reference in chapter 246-290 WAC, Group A Public Water Supplies, and chapter 246-390 WAC, Drinking Water Laboratory Certification and Data Reporting. The Department of Health (department) amended WAC 246-290-002, 246-290-010, 246-290-025, 246-290-300, 246-290-310, 246-290-320, 246-290-71002, 246-290-72004, 246-290-72012, 246-390-010 and 246-290-075, to align these sections with the recent changes to 40 CFR Part 141 regarding PFAS. The amendments to chapters 246-290 and 246-390 WAC incorporate by reference the requirements for monitoring, reporting, public notification, treatment, and violations, as well as incorporate the federal PFAS Maximum Contaminant Levels into the appropriate contaminant tables in rule. The amendments also include minor editorial changes and definition updates to ensure consistency of terms between the federal and state rules.

Citation of rules affected by this order:

New: None

Repealed: None

Amended: WAC 246-290-002, 246-290-010, 246-290-025, 246-290-300, 246-290-310, 246-290-320, 246-290-71002, 246-290-72004, 246-290-72012, 246-390-010, and 246-390-075.

Suspended: None

Statutory authority for adoption: RCW 43.20.050 and RCW 70A.125.080

Other authority: None

PERMANENT RULE (Including Expedited Rule Making)

Adopted under notice filed as WSR 25-09-150 on 04/22/2025 (date).

Describe any changes other than editing from proposed to adopted version: None.

If a preliminary cost-benefit analysis was prepared under RCW 34.05.328, a final cost-benefit analysis is available by contacting: N/A

Name:

Address:

Phone:

Fax:

TTY:

Email:

Web site:

Other:

Note: If any category is left blank, it will be calculated as zero. No descriptive text.

Count by whole WAC sections only, from the WAC number through the history note. A section may be counted in more than one category.

The number of sections adopted in order to comply with:

Federal statute:	New	0	Amended	0	Repealed	0
Federal rules or standards:	New	0	Amended	11	Repealed	0
Recently enacted state statutes:	New	0	Amended	0	Repealed	0

The number of sections adopted at the request of a nongovernmental entity:

	New	0	Amended	0	Repealed	0

The number of sections adopted on the agency's own initiative:

	New	0	Amended	0	Repealed	0

The number of sections adopted in order to clarify, streamline, or reform agency procedures:

	New	0	Amended	0	Repealed	0

The number of sections adopted using:

Negotiated rule making:	New	0	Amended	0	Repealed	0
Pilot rule making:	New	0	Amended	0	Repealed	0
Other alternative rule making:	New	0	Amended	11	Repealed	0

Date Adopted: 6/17/2025

Name: Kristin Peterson, JD for Jessica Todorovich, MS

Title: Chief of Policy for Acting Secretary of Health

Signature:



AMENDATORY SECTION (Amending WSR 17-01-062, filed 12/14/16, effective 1/14/17)

WAC 246-290-002 Guidance. (1) The department has numerous guidance documents available to help purveyors comply with state and federal rules regarding drinking water. These include documents on the following subjects:

(a) Compliance;
(b) Consumer and public education;
(c) Contaminants;
(d) Cross-connection control and backflow prevention;
(e) Emergency response and drinking water security;
(f) Engineering design and water treatment;
(g) Financial assistance and state revolving fund (SRF);
(h) General information;
(i) Groundwater protection;
(j) Growth management;
(k) Operations and maintenance;
(l) Operator certification;
(m) Planning and financial viability;
(n) Regulations;
(o) Small water systems;
(p) System approval;
(q) Water quality monitoring and source protection;
(r) Water system planning; and
(s) Water use efficiency.

(2) The department's guidance documents are available online at ((~~https://fortress.wa.gov/doh/eh/dw/publications/publications.cfm~~)) https://fortress.wa.gov/doh/odwpubs/publications/ or through U.S. mail at P.O. Box 47822, Olympia, Washington 98504-7822.

(3) Federal guidance documents are available from the Environmental Protection Agency (EPA) for a wide range of topics. These are available from the EPA Office of Ground Water and Drinking Water website at http://water.epa.gov/drink.index.cfm.

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-010 Definitions, abbreviations, and acronyms. The definitions in this section apply throughout this chapter unless the context clearly indicates otherwise.

(1) "Acute" means posing an immediate risk to human health.

(2) "Adverse effect" means a biological change, functional impairment, or pathologic lesion that may affect the performance of the whole organism or reduce an organism's ability to respond to an additional environmental challenge.

(3) "Alternative filtration technology" means a filtration process for substantial removal of particulates (generally > 2-log *Giardia lamblia* cysts and ≥ 2-log removal of *Cryptosporidium* oocysts) by other than conventional, direct, diatomaceous earth, or slow sand filtration processes.

(4) "Analogous treatment system" means an existing water treatment system that has unit processes and source water quality characteristics that are similar to a proposed treatment system.

(5) "ANSI" means the American National Standards Institute.

(6) "Approved air gap (AG)" means a physical separation between the free-flowing end of a potable water supply pipeline and the overflow rim of an open or nonpressurized receiving vessel.

To be an air gap approved by the department, the separation must be at least:

(a) Twice the diameter of the supply piping measured vertically from the overflow rim of the receiving vessel, and in no case be less than one inch, when unaffected by vertical surfaces (sidewalls); and

(b) Three times the diameter of the supply piping, if the horizontal distance between the supply pipe and a vertical surface (sidewall) is less than or equal to three times the diameter of the supply pipe, or if the horizontal distance between the supply pipe and intersecting vertical surfaces (sidewalls) is less than or equal to four times the diameter of the supply pipe and in no case less than one and one-half inches.

(7) "Approved atmospheric vacuum breaker (AVB)" means an AVB of make, model, and size that is approved by the department. AVBs that appear on the current approved backflow prevention assemblies list developed by the University of Southern California Foundation for Cross-Connection Control and Hydraulic Research or that are listed or approved by other nationally recognized testing agencies (such as IAPMO, ANSI, or UL) acceptable to the authority having jurisdiction are considered approved by the department.

(8) "Approved backflow preventer" means an approved air gap, an approved backflow prevention assembly, or an approved AVB. The terms "approved backflow preventer," "approved air gap," or "approved backflow prevention assembly" refer only to those approved backflow preventers relied upon by the purveyor for the protection of the public water system. The requirements of WAC 246-290-490 do not apply to backflow preventers installed for other purposes.

(9) "Approved backflow prevention assembly" means an RPBA, RPDA, DCVA, DCDA, PVBA, or SVBA of make, model, and size that is approved by the department. Assemblies that appear on the current approved backflow prevention assemblies list developed by the University of Southern California Foundation for Cross-Connection Control and Hydraulic Research or other entity acceptable to the department are considered approved by the department.

(10) "As-built drawing" means the drawing created by an engineer from the collection of the original design plans, including changes made to the design or to the system, that reflects the actual constructed condition of the water system.

(11) "Assessment source water monitoring" means an evaluation of groundwater sources that may be at risk for fecal contamination. Assessment source water monitoring involves the collection of source water samples at regular intervals and analysis of those samples for fecal indicators as directed by the department.

(12) "Authority having jurisdiction" (formerly known as local administrative authority) means the local official, board, department, or agency authorized to administer and enforce the provisions of the Uniform Plumbing Code as adopted under chapter 19.27 RCW.

(13) "Authorized agent" means any person who:

(a) Makes decisions regarding the operation and management of a public water system whether or not he or she is engaged in the physical operation of the system;

(b) Makes decisions whether to improve, expand, purchase, or sell the system; or

(c) Has discretion over the finances of the system.

(14) "Authorized consumption" means the volume of metered and unmetered water used for municipal water supply purposes by consumers, the purveyor, and others authorized to do so by the purveyor including, but not limited to, firefighting and training, flushing of mains and sewers, street cleaning, and watering of parks and landscapes. These volumes may be billed or unbilled.

(15) "Average day demand (ADD)" means the total quantity of water use from all sources of supply as measured or estimated over a calendar year divided by ((~~three hundred sixty-five~~)) 365. ADD is typically expressed as gallons per day (gpd) per equivalent residential unit (ERU).

(16) "AWWA" means the American Water Works Association.

(17) "Backflow" means the undesirable reversal of flow of water or other substances through a cross-connection into the public water system or consumer's potable water system.

(18) "Backflow assembly tester (BAT)" means a person holding a valid BAT certificate issued under chapter 246-292 WAC.

(19) "Backpressure" means a pressure (caused by a pump, elevated tank or piping, boiler, or other means) on the consumer's side of the service connection that is greater than the pressure provided by the public water system and which may cause backflow.

(20) "Backsiphonage" means backflow due to a reduction in system pressure in the purveyor's distribution system and/or consumer's water system.

(21) "Bag filter" means a pressure-driven separation device that removes particulate matter larger than 1 micrometer using an engineered porous filtration media. They are typically constructed of a nonrigid, fabric filtration media housed in a pressure vessel in which the direction of flow is from the inside of the bag to outside.

(22) "Bank filtration" means a water treatment process that uses a well to recover surface water that has naturally infiltrated into groundwater through a riverbed or bank(s). Infiltration is typically enhanced by the hydraulic gradient imposed by a nearby pumping water supply or other well(s).

(23) "Best available technology" means the best technology, treatment techniques, or other means that EPA finds, after examination for efficacy under field conditions, are available, taking cost into consideration.

(24) "Bioaccumulative" means a chemical that can accumulate in the body when regular exposure occurs through drinking water.

(25) "Blended sample" means a sample collected from two or more individual sources at a point downstream of the confluence of the individual sources and prior to the first connection.

(26) "C" means the residual disinfectant concentration in mg/L at a point before or at the first consumer.

(27) "Cartridge filter" means a pressure-driven separation device that removes particulate matter larger than 1 micrometer using an engineered porous filtration media. They are typically constructed as rigid or semi-rigid, self-supporting filter elements housed in pressure vessels in which flow is from the outside of the cartridge to the inside.

(28) "Category red operating permit" means an operating permit identified under chapter 246-294 WAC. Placement in this category results in permit issuance with conditions and a determination that the system is inadequate.

(29) "C.F.R." means the Code of Federal Regulations.

(30) "Chemical contaminant treatment facility" means a treatment facility specifically used for the purpose of removing chemical contaminants.

(31) "Clarification" means a treatment process that uses gravity (sedimentation) or dissolved air (flotation) to remove flocculated particles.

(32) "Clean compliance history" means a record of:

(a) No *E. coli* MCL violations;

(b) No monitoring violations under WAC 246-290-300(3); and

(c) No coliform treatment technique trigger exceedances or treatment technique violations under WAC 246-290-320(2) or 246-290-415.

(33) "Closed system" means any water system or portion of a water system in which water is transferred to a higher-pressure zone closed to the atmosphere, such as when no gravity storage is present.

(34) "Coagulant" means a chemical used in water treatment to destabilize particulates and accelerate the rate at which they aggregate into larger particles.

(35) "Coagulation" means a process using coagulant chemicals and rapid mixing to destabilize colloidal and suspended particles and agglomerate them into flocs.

(36) "Combination fire protection system" means a fire sprinkler system that:

(a) Is supplied only by the purveyor's water;

(b) Does not have a fire department pumper connection; and

(c) Is constructed of approved potable water piping and materials that serve both the fire sprinkler system and the consumer's potable water system.

(37) "Combined distribution system" means the interconnected distribution system consisting of the distribution systems of wholesale systems and of the consecutive systems that receive finished water.

(38) "Completely treated water" means water from a surface water source, or a groundwater source under the direct influence of surface water (GWI) source that receives filtration or disinfection treatment that fully complies with the treatment technique requirements of Part 6 of this chapter as determined by the department.

(39) "Composite correction program (CCP)" means a program that consists of two elements - a comprehensive performance evaluation (CPE) and comprehensive technical assistance (CTA).

(40) "Composite sample" means a sample in which more than one source is sampled individually by the water system and then composited by a certified laboratory by mixing equal parts of water from each source (up to five different sources) and then analyzed as a single sample.

(41) "Comprehensive monitoring plan" means a schedule that describes both the frequency and appropriate locations for sampling of drinking water contaminants as required by state and federal rules.

(42) "Comprehensive performance evaluation (CPE)" means a thorough review and analysis of a treatment plant's performance-based capabilities and associated administrative, operation and maintenance practices. It is conducted to identify factors that may be adversely impacting a plant's capability to achieve compliance and emphasizes

approaches that can be implemented without significant capital improvements.

The comprehensive performance evaluation must consist of at least the following components:

(a) Assessment of plant performance;

(b) Evaluation of major unit processes;

(c) Identification and prioritization of performance limiting factors;

(d) Assessment of the applicability of comprehensive technical assistance; and

(e) Preparation of a CPE report.

(43) "Comprehensive technical assistance (CTA)" means the performance improvement phase that is implemented if the CPE results indicate improved performance potential. The system must identify and systematically address plant-specific factors. The CTA is a combination of using CPE results as a basis for follow-up, implementing process control priority-setting techniques, and maintaining long-term involvement to systematically train staff and administrators.

(44) "Confirmation" means to demonstrate that the result of a sample accurately represents the original sample result by analyzing another sample from the same location within a reasonable given period of time.

(45) "Confluent growth" means a continuous bacterial growth covering a portion or the entire filtration area of a membrane filter in which bacterial colonies are not discrete.

(46) "Consecutive system" means a public water system that receives some or all of its finished water from one or more wholesale systems. Delivery may be through a direct connection or through the distribution system of one or more consecutive systems.

(47) "Construction completion report" means a form provided by the department and completed for each specific construction project to document:

(a) Project construction in accordance with this chapter and general standards of engineering practice;

(b) Physical capacity changes; and

(c) Satisfactory test results.

The completed form must be stamped with an engineer's seal and signed and dated by a professional engineer.

(48) "Consumer" means any person receiving water from a public water system from either the meter, or the point where the service line connects with the distribution system if no meter is present. For purposes of cross-connection control, "consumer" means the owner or operator of a water system connected to a public water system through a service connection.

(49) "Consumer's water system," as used in WAC 246-290-490, means any potable or industrial water system that begins at the point of delivery from the public water system and is located on the consumer's premises. The consumer's water system includes all auxiliary sources of supply, storage, treatment, and distribution facilities, piping, plumbing, and fixtures under the control of the consumer.

(50) "Contaminant" means a substance present in drinking water that may adversely affect the health of the consumer or the aesthetic qualities of the water.

(51) "Contingency plan" means that portion of the wellhead protection program section of the water system plan or small water system management program that addresses the replacement of the major well(s) or wellfield in the event of loss due to groundwater contamination.

(52) "Continuous monitoring" means determining water quality with automatic recording analyzers that operate without interruption ((~~twenty-four~~)) 24 hours per day.

(53) "Conventional filtration treatment" means a series of processes including coagulation, flocculation, clarification, and filtration that together result in substantial particulate removal in compliance with Part 6 of this chapter.

(54) "Corrective action plan" means specific written actions and deadlines developed by the water system or the department that the system must follow as a result of either the identification of significant deficiencies during a sanitary survey or the determination of a fecal indicator-positive sample in source water monitoring.

(55) "Cost-effective" means the benefits exceed the costs.

(56) "Council" means the Washington state building code council under WAC 51-04-015(2).

(57) "Critical water supply service area (CWSSA)" means a geographical area which is characterized by a proliferation of small, inadequate water systems, or by water supply problems which threaten the present or future water quality or reliability of service in a manner that efficient and orderly development may best be achieved through coordinated planning by the water utilities in the area as set forth by the Public Water System Coordination Act, chapter 70A.100 RCW and chapter 246-293 WAC.

(58) "Cross-connection" means any actual or potential physical connection between a public water system or the consumer's water system and any source of nonpotable liquid, solid, or gas that could contaminate the potable water supply by backflow.

(59) "Cross-connection control program" means the administrative and technical procedures the purveyor implements to protect the public water system from contamination via cross-connections as required in WAC 246-290-490.

(60) "Cross-connection control specialist (CCS)" means a person holding a valid CCS certificate issued under chapter 246-292 WAC.

(61) "Cross-connection control summary report" means the annual report that describes the status of the purveyor's cross-connection control program.

(62) "CT" or "CTcalc" means the product of "residual disinfectant concentration" (C) and the corresponding "disinfectant contact time" (T) i.e., "C" x "T."

(63) "$CT_{99.9}$" means the CT value required for 99.9 percent (3-log) inactivation of *Giardia lamblia* cysts.

(64) "CTreq" means the CT value a system shall provide to achieve a specific percent inactivation of *Giardia lamblia* cysts or other pathogenic organisms of health concern as directed by the department.

(65) "Curtailment" means short-term, infrequent actions by a purveyor and its consumers to reduce their water use during or in anticipation of a water shortage.

(66) "DBPs" means disinfection byproducts.

(67) "DCDA" means a double check detector assembly.

(68) "DCVA" means a double check valve assembly.

(69) "Dead storage" means the volume of stored water not available to all consumers at the minimum design pressure under WAC 246-290-230 (5) and (6).

(70) "Demand forecast" means an estimate of future water system water supply needs assuming historically normal weather conditions and calculated using numerous parameters, including population, historic

water use, local land use plans, water rates and their impacts on consumption, employment, projected water use efficiency savings from implementation of a water use efficiency program, and other appropriate factors.

(71) "Department" means the Washington state department of health or health officer as identified in a joint plan of responsibility under WAC 246-290-030(1).

(72) "Design and construction standards" means department design guidance and other peer reviewed documents generally accepted by the engineering profession as containing fundamental criteria for design and construction of water facility projects. Design and construction standards are comprised of performance and sizing criteria and reference general construction materials and methods.

(73) "Detectable residual disinfectant concentration" means 0.2 mg/L free chlorine, total chlorine, combined chlorine, or chlorine dioxide.

(74) "Diatomaceous earth filtration" means a filtration process for substantial removal of particulates (> 2-log *Giardia lamblia* cysts) in which:

(a) A precoat cake of graded diatomaceous earth filter media is deposited on a support membrane (septum); and

(b) Water is passed through the cake on the septum while additional filter media, known as body feed, is continuously added to the feed water to maintain the permeability of the filter cake.

(75) "Direct filtration" means a series of processes including coagulation, flocculation, and filtration (but excluding sedimentation) that together result in substantial particulate removal in compliance with Part 6 of this chapter.

(76) "Direct service connection" means a service hookup to a property that is contiguous to a water distribution main and where additional distribution mains or extensions are not needed to provide service.

(77) "Disinfectant contact time (T in CT)" means:

(a) When measuring the first or only C, the time in minutes it takes water to move from the point of disinfectant application to a point where the C is measured; and

(b) For subsequent measurements of C, the time in minutes it takes water to move from one C measurement point to the C measurement point for which the particular T is being calculated.

(78) "Disinfection" means the use of chlorine or other agent or process the department approves for killing or inactivating microbiological organisms, including pathogenic and indicator organisms.

(79) "Disinfection profile" means a summary of *Giardia lamblia* inactivation through a surface water treatment plant.

(80) "Distribution coliform sample" means a sample of water collected from a representative location in the distribution system at or after the first service and analyzed for coliform presence in compliance with this chapter.

(81) "Distribution-related projects" means distribution projects such as storage tanks, booster pump facilities, transmission mains, pipe linings, and tank coating. It does not mean source of supply (including interties) or water quality treatment projects.

(82) "Distribution system" means all piping components of a public water system that serve to convey water from transmission mains linked to source, storage and treatment facilities to the consumer excluding individual services.

(83) "Domestic or other nondistribution system plumbing problem" means contamination of a system having more than one service connection with the contamination limited to the specific service connection from which the sample was taken.

(84) "Dual sample set" means a set of two samples collected at the same time and same location, with one sample analyzed for TTHM and the other sample analyzed for HAA5. Dual sample sets are collected for the purposes of conducting an IDSE under WAC 246-290-300 (6)(b)(i)(F) and determining compliance with the TTHM and HAA5 MCLs under WAC 246-290-310(4).

(85) "Duplicate (verification) sample" means a second sample collected at the same time and location as the first sample and used for verification.

(86) "DVGW" means Deutsche Vereinigung des Gas und Wasserfaches.

(87) "Elected governing board" means the elected officers with ultimate legal responsibility for operational, technical, managerial, and financial decisions for a public water system.

(88) "Emergency" means an unforeseen event that causes damage or disrupts normal operations and requires immediate action to protect public health and safety.

(89) "Emergency source" means any source that a purveyor intends to use for emergency purposes only and not used for routine or seasonal water demands.

(90) "Engineering design review report" means a form provided by the department and completed for a specific distribution-related project to document:

(a) Engineering review of a project report and/or construction documents under the submittal exception process in WAC 246-290-125(3); and

(b) Design in accordance with this chapter and general standards of engineering practice.

(c) The completed form must be stamped with engineer's seal, and signed and dated by a professional engineer.

(91) "EPA" means the U.S. Environmental Protection Agency.

(92) "Equalizing storage" means the volume of storage needed to supplement supply to consumers when the peak hourly demand exceeds the total source pumping capacity.

(93) "Equivalent residential unit (ERU)" means a system-specific unit of measure used to express the amount of water consumed by a typical full-time single family residence.

(94) "Expanding public water system" means a public water system that increases the geographical area where direct service connections are available or increases the approved number of service connections.

(95) "Filter profile" means a graphical representation of individual filter performance in a direct or conventional surface water filtration plant, based on continuous turbidity measurements or total particle counts versus time for an entire filter run, from startup to backwash inclusively, that includes an assessment of filter performance while another filter is being backwashed.

(96) "Filtration" means a process for removal of particulate matter from water by passage through porous media.

(97) "Financial viability" means the capability of a water system to obtain sufficient funds to construct, operate, maintain, and manage a public water system, on a continuing basis, in full compliance with federal, state, and local requirements.

(98) "Finished water" means water introduced into a public water system's distribution system and is intended for distribution and con-

sumption without further treatment, except as treatment necessary to maintain water quality in the distribution system (e.g., booster disinfection, addition of corrosion control chemicals).

(99) "Finished water storage facility" means a water storage structure that is integrated with a water system's distribution network to provide for variable system demands including, but not limited to, daily equalizing storage, standby storage, or fire reserves, or to provide for disinfectant contact time.

(100) "Fire flow" means the maximum rate and duration of water flow needed to suppress a fire under WAC 246-293-640 or as required under local fire protection authority standards.

(101) "Fire suppression storage" means the volume of stored water available during fire suppression activities to satisfy minimum pressure requirements per WAC 246-290-230.

(102) "First consumer" means the first service connection associated with any source (i.e., the point where water is first withdrawn for human consumption, excluding connections where water is delivered to another water system covered by these regulations).

(103) "Flocculation" means a process enhancing agglomeration and collection of colloidal and suspended particles into larger, more easily settleable or filterable particles by gentle stirring.

(104) "Flowing stream" means a course of running water flowing in a definite channel.

(105) "Flow-through fire protection system" means a fire sprinkler system that:

(a) Is supplied only by the purveyor's water;

(b) Does not have a fire department pumper connection;

(c) Is constructed of approved potable water piping and materials to which sprinkler heads are attached; and

(d) Terminates at a connection to a toilet or other plumbing fixture to prevent stagnant water.

(106) "Forecasted demand characteristics" means the factors that may affect a public water system's projected water needs.

(107) "Future service area" means a specific area a water system in a CWSSA plans to provide water service as determined by a written agreement between purveyors under chapter 70A.100 RCW and chapter 246-293 WAC.

(108) "GAC" means granular activated carbon.

(109) "GAC10" means granular activated carbon filter beds with an empty-bed contact time of ((~~ten~~)) 10 minutes based on average daily flow and a carbon reactivation frequency of every ((~~one hundred eighty~~)) 180 days, except that the reactivation frequency for GAC10 used as a best available technology for compliance with MCLs under WAC 246-290-310(4) shall be ((~~one hundred twenty~~)) 120 days.

(110) "GAC20" means granular activated carbon filter beds with an empty-bed contact time of ((~~twenty~~)) 20 minutes based on average daily flow and a carbon reactivation frequency of every ((~~two hundred forty~~)) 240 days.

(111) "Governing body" means the individual or group of individuals with ultimate legal responsibility for operational, technical, managerial, and financial decisions for a public water system.

(112) "gph" means gallons per hour.

(113) "gpm" means gallons per minute.

(114) "Grab sample" means a water quality sample collected at a specific instant in time and analyzed as an individual sample.

(115) "Groundwater system" means all public water systems that use groundwater including:

(a) Consecutive systems receiving finished groundwater; or

(b) Surface water systems with groundwater sources except those systems that combine all sources prior to treatment.

(116) "Groundwater under the direct influence of surface water (GWI)" means any water beneath the surface of the ground that the department determines has the following characteristics:

(a) Significant occurrence of insects or other macroorganisms, algae, or large-diameter pathogens such as *Giardia lamblia* or, *Cryptosporidium*; or

(b) Significant and relatively rapid shifts in water characteristics such as turbidity, temperature, conductivity, or pH closely correlating to climatological or surface water conditions where natural conditions cannot prevent the introduction of surface water pathogens into the source at the system's point of withdrawal.

(117) "Guideline" means a department document assisting the purveyor in meeting a rule requirement.

(118) "GWR" means groundwater rule.

(119) "HAA5" means haloacetic acids (five).

(120) "Hazard index (HI)" is the sum of component hazard quotients (HQs), which are calculated by dividing the measured regulated PFAS component concentration in water (e.g., expressed as parts per trillion (ppt) or nanograms per liter (ng/l)) by the associated health-based water concentration (HBWC) expressed in the same units as the measured concentration (e.g., ppt or ng/l). For PFAS, a mixture hazard index greater than 1 (unitless) is an exceedance of the MCL.

(121) "Hazard quotient (HQ)" means the ratio of the measured concentration in drinking water to the health-based water concentration (HBWC).

(122) "Health-based water concentration (HBWC)" means level below which there are no known or anticipated adverse health effects over a lifetime of exposure, including sensitive populations and life stages, and allows for an adequate margin of safety.

(123) "Health officer" means the health officer of the city, county, city-county health department or district, or an authorized representative.

((~~(121)~~)) (124) "Heterotrophic plate count (HPC)" means a procedure to measure a class of bacteria that use organic nutrients for growth. The density of these bacteria in drinking water is measured as colony forming units per milliliter and is referred to as the HPC.

((~~(122)~~)) (125) "HFPO-DA or GenX chemicals" means Chemical Abstract Service registration number 122499-17-6, chemical formula C6F11O3-, International Union of Pure and Applied Chemistry preferred name 2,3,3,3-tetrafluoro-2- (heptafluoropropoxy)propanoate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

(126) "High health cross-connection hazard" means a cross-connection involving any substance that could impair the quality of potable water and create an actual public health hazard through injury, poisoning, or spread of disease.

((~~(123)~~)) (127) "Human consumption" means the use of water for drinking, bathing or showering, hand washing, food preparation, cooking, or oral hygiene.

((~~(124)~~)) (128) "Hydraulic analysis" means the study of a water system's distribution main and storage network to determine present or future adequacy for provision of service to consumers within the established design parameters for the system under peak flow conditions, including fire flow. The analysis is used to establish any need for

improvements to existing systems or to substantiate adequacy of design for distribution system components such as piping, elevated storage, booster stations or similar facilities used to pump and convey water to consumers.

((~~(125)~~)) (129) "IAPMO" means the International Association of Plumbing and Mechanical Officials.

((~~(126)~~)) (130) "IDSE" means an initial distribution system evaluation.

((~~(127)~~)) (131) "Inactivation" means a process which renders pathogenic microorganisms incapable of producing disease.

((~~(128)~~)) (132) "Inactivation ratio" means the ratio obtained by dividing CTcalc by CTreq.

((~~(129)~~)) (133) "Incompletely treated water" means water from a surface or GWI source that receives filtration and/or disinfection treatment that does not fully comply with the treatment technique requirements of Part 6 of this chapter as determined by the department.

((~~(130)~~)) (134) "In-line filtration" means a series of processes, including coagulation and filtration (but excluding flocculation and sedimentation) that together result in particulate removal.

((~~(131)~~)) (135) "In-premises protection" means a method of protecting the health of consumers served by the consumer's potable water system, located within the property lines of the consumer's premises by the installation of an approved air gap or backflow prevention assembly at the point of hazard, which is generally a plumbing fixture.

((~~(132)~~)) (136) "Intertie" means an interconnection between public water systems permitting the exchange or delivery of water between those systems.

((~~(133)~~)) (137) "kPa" means kilo pascal (SI units of pressure).

((~~(134)~~)) (138) "Lake or reservoir" means a natural or man-made basin or hollow on the earth's surface in which water collects or is stored that may or may not have a current or single direction of flow.

((~~(135)~~)) (139) "Legionella" means a genus of bacteria containing species which cause a type of pneumonia called Legionnaires' disease.

((~~(136)~~)) (140) "Level 1 assessment" means an evaluation to identify the possible presence of sanitary defects, defects in distribution system coliform monitoring practices, and when possible, the likely reason that the system triggered the assessment. The assessment is conducted by the system operator or the purveyor.

((~~(137)~~)) (141) "Level 2 assessment" means an evaluation to identify the possible presence of sanitary defects, defects in distribution system coliform monitoring practices, and when possible, the likely reason that the system triggered the assessment. A level 2 assessment is a more detailed examination of the system (including the system's monitoring and operational practices) than is a level 1 assessment through the use of a more comprehensive investigation and review of available information, additional internal and external resources, and other relevant practices. The level 2 assessment is conducted by a party approved by the department.

((~~(138)~~)) (142) "Limited alternative to filtration" means a process that ensures greater removal and/or inactivation efficiencies of pathogenic organisms than would be achieved by the combination of filtration and chlorine disinfection.

((~~(139)~~)) (143) "Local plans and regulations" means any comprehensive plan or development regulation adopted under chapter 36.70A RCW or any other applicable comprehensive plan, land use plan, or development regulation adopted by a city, town, or county for the applicable service area.

((~~(140)~~)) (144) "Locational running annual average (LRAA)" means the average of sample analytical results for samples taken at a particular monitoring location during the previous four calendar quarters.

((~~(141)~~)) (145) "Low cross-connection hazard" means a cross-connection that could impair the quality of potable water to a degree that does not create a hazard to the public health, but does adversely and unreasonably affect the aesthetic qualities of potable waters for domestic use.

((~~(142)~~)) (146) "Major project" means all construction projects subject to the State Environmental Policy Act (SEPA) under chapter 43.21C RCW, and meeting the requirements of WAC 246-03-030 (3)(a).

((~~(143)~~)) (147) "Mandatory curtailment" means curtailment required by a public water system of specified water uses and consumer classes for a specified period of time.

((~~(144)~~)) (148) "Marginal costs" means the costs incurred by producing the next increment of supply.

((~~(145)~~)) (149) "Maximum contaminant level (MCL)" means the maximum permissible level of a contaminant in water the purveyor delivers to any public water system user, measured at the locations identified under WAC 246-290-310, Table 5.

((~~(146)~~)) (150) "Maximum contaminant level violation" means a confirmed measurement above the MCL and for a duration of time, where applicable, as outlined under WAC 246-290-310.

((~~(147)~~)) (151) "Maximum day demand (MDD)" means the highest actual or estimated quantity of water that is, or is expected to be, used over a ((~~twenty-four~~)) 24 hour period, excluding unusual events or emergencies. MDD is typically expressed as gallons per day per ERU (gpd/ERU).

((~~(148)~~)) (152) "Membrane filtration" means a pressure or vacuum driven separation process in which particulate matter larger than 1 micrometer is rejected by an engineered barrier, primarily through a size-exclusion mechanism, and which has a measurable removal efficiency of a target organism that can be verified through the application of a direct integrity test. This definition includes the common membrane technologies of microfiltration, ultrafiltration, nanofiltration, and reverse osmosis.

((~~(149)~~)) (153) "Metabolite" means a byproduct of a contaminant in drinking water formed during a natural biological process in the body.

((~~(150)~~)) (154) "mg/L" means milligrams per liter (1 mg/L = 1 ppm).

((~~(151)~~)) (155) "mL" means a milliliter.

((~~(152)~~)) (156) "mm" means a millimeter.

((~~(153)~~)) (157) "Monitoring waiver" means an action taken by the department under WAC 246-290-300 (4)(g), (7)(f), or (10)(h) to allow a water system to reduce specific monitoring requirements based on a determination of low source vulnerability to contamination.

((~~(154)~~)) (158) "MRDL" means the maximum residual disinfectant level.

((~~(155)~~)) (159) "MRDLG" means the maximum residual disinfectant level goal.

((~~(156)~~)) (160) "MTTP" means maximum total trihalomethane potential.

((~~(157)~~)) (161) "Municipal water supplier" means an entity that supplies water for municipal water supply purposes.

((~~(158)~~)) (162) "Municipal water supply purposes" means a beneficial use of water:

(a) For residential purposes through ((~~fifteen~~)) 15 or more residential service connections or for providing residential use of water for a nonresidential population that is, on average, at least ((~~twenty-five~~)) 25 people for at least ((~~sixty~~)) 60 days a year;

(b) For governmental or governmental proprietary purposes by a city, town, public utility district, county, sewer district, or water district; or

(c) Indirectly for the purposes in (a) or (b) of this definition through the delivery of treated or raw water to a public water system for such use.

(i) If water is beneficially used under a water right for the purposes listed in (a), (b), or (c) of this definition, any other beneficial use of water under the right generally associated with the use of water within a municipality is also for "municipal water supply purposes," including, but not limited to, beneficial use for commercial, industrial, irrigation of parks and open spaces, institutional, landscaping, fire flow, water system maintenance and repair, or related purposes.

(ii) If a governmental entity holds a water right that is for the purposes listed in (a), (b), or (c) of this definition, its use of water or its delivery of water for any other beneficial use generally associated with the use of water within a municipality is also for "municipal water supply purposes," including, but not limited to, beneficial use for commercial, industrial, irrigation of parks and open spaces, institutional, landscaping, fire flow, water system maintenance and repair, or related purposes.

((~~(159)~~)) (163) "Nested storage" means one component of storage is contained within the component of another.

((~~(160)~~)) (164) "ng/L" means nanograms per liter.

((~~(161)~~)) (165) "Nonacute" means posing a possible or less than immediate risk to human health.

((~~(162)~~)) (166) "Nonresident" means a person having access to drinking water from a public water system who lives elsewhere. Examples include travelers, transients, employees, students, etc.

((~~(163)~~)) (167) "Normal operating conditions" means those conditions associated with the designed, day-to-day provision of potable drinking water that meets regulatory water quality standards and the routine service expectations of the system's consumers at all times, including meeting fire flow demands. Operation under conditions such as power outages, floods, or unscheduled transmission or distribution disruptions, even if considered in the system design, are considered abnormal.

((~~(164)~~)) (168) "NSF" means NSF International (formerly known as the National Sanitation Foundation (NSF)).

((~~(165)~~)) (169) "NTNC" means nontransient noncommunity.

((~~(166)~~)) (170) "NTU" means a nephelometric turbidity unit.

((~~(167)~~)) (171) "ONORM" means Osterreichisches Normungsinstitut.

((~~(168)~~)) (172) "Operational storage" means the volume of distribution storage associated with source or booster pump normal cycling times under normal operating conditions and is additive to the equalizing and standby storage components, and to fire flow storage if this storage component exists for any given tank.

((~~(169)~~)) (173) "pCi/L" means picocuries per liter.

((~~(170)~~)) (174) "PFAS" means per- and polyfluoroalkyl substances, a group of man-made chemicals found in products such as aqueous film-

forming foam used to suppress petroleum-based fires, nonstick cookware, stain-resistant fabrics and many other products, and as defined in RCW 70A.350.010(8).

((~~(171)~~)) (175) "PFBS" means ((~~perfluorobutane sulfonic acid~~)) Chemical Abstract Service registration number 45187-15-3, chemical formula C4F9SO3-, perfluorobutane sulfonate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

((~~(172)~~)) (176) "PFHxS" means ((~~perfluorohexane sulfonic acid~~)) Chemical Abstract Service registration number 108427-53-8, chemical formula C6F13SO3-, perfluorohexane sulfonate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

((~~(173)~~)) (177) "PFNA" means ((~~perfluorononanoic acid~~)) Chemical Abstract Service registration number 72007-68-2, chemical formula C9F17O2-, perfluorononanoate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

((~~(174)~~)) (178) "PFOA" means ((~~perfluorooctanoic acid, also known as C8~~)) Chemical Abstract Service registration number 45285-51-6, chemical formula C8F15O2-, perfluorooctanoate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

((~~(175)~~)) (179) "PFOS" means ((~~perfluorooctane sulfonic acid~~)) Chemical Abstract Service registration number 45298-90-6, chemical formula C8F17SO3-, perfluorooctanesulfonate, along with its conjugate acid and any salts, derivatives, isomers, or combinations thereof.

((~~(176)~~)) (180) "Peak hourly demand (PHD)" means the maximum rate of water use, excluding fire flow, that can be expected to occur within a defined service area over a continuous ((~~sixty~~)) 60 minute time period. PHD is typically expressed in gallons per minute (gpm).

((~~(177)~~)) (181) "Peak hourly flow" means, for the purpose of CT calculations, the greatest volume of water passing through the system during any one hour in a day.

((~~(178)~~)) (182) "Performance criteria" means the level at which a system shall operate in order to maintain system reliability compliance, in accordance with WAC 246-290-420, and to meet consumers' reasonable expectations.

((~~(179)~~)) (183) "Permanent residence" means any dwelling that is, or could reasonably be expected to be, occupied on a continuous basis.

((~~(180)~~)) (184) "Permanent source" means a public water system supply source that is used regularly each year, and based on expected operational requirements of the system, will be used more than three consecutive months in any ((~~twelve~~)) 12-month period. For seasonal water systems that are in operation for less than three consecutive months per year, their sources are also considered to be permanent.

((~~(181)~~)) (185) "Plant intake" means the works or structures at the head of a conduit through which water is diverted from a source (e.g., river or lake) into the treatment plant.

((~~(182)~~)) (186) "Point of disinfectant application" means the point where the disinfectant is added, and where water downstream of that point is not subject to contamination by untreated surface water.

((~~(183)~~)) (187) "Population served" means the number of persons, resident and nonresident, having immediate access to drinking water from a public water system, whether or not persons have actually consumed water from that system. The number of nonresidents is the average number of persons having immediate access to drinking water on days access was provided during that month. In the absence of specific population data, the number of residents is computed by multiplying the number of active services by two and one-half.

((~~(184)~~)) (188) "Potable" means water suitable for drinking by the public.

((~~(185)~~)) (189) "Potential GWI" means a source identified by the department as possibly under the influence of surface water, and includes, but is not limited to, all wells with a screened interval ((~~fifty~~)) 50 feet or less from the ground surface at the wellhead and located within ((~~two hundred~~)) 200 feet of a surface water, and all Ranney wells, infiltration galleries, and springs.

((~~(186)~~)) (190) "ppm" means parts per million (1 ppm = 1 mg/L).

((~~(187)~~)) (191) "ppt" means parts per trillion (1 ppt = 1 ng/L).

((~~(188)~~)) (192) "Premises isolation" means a method of protecting a public water system by installation of approved air gaps or approved backflow prevention assemblies at or near the service connection or alternative location acceptable to the purveyor to isolate the consumer's water system from the purveyor's distribution system.

((~~(189)~~)) (193) "Presedimentation" means a preliminary treatment process used to remove gravel, sand, and other particulate material from the source water through settling before the water enters the primary clarification and filtration processes in a treatment plant.

((~~(190)~~)) (194) "Pressure filter" means an enclosed vessel containing properly sized and graded granular media through which water is forced under greater than atmospheric pressure.

((~~(191)~~)) (195) "Primary disinfection" means a treatment process for achieving inactivation of *Giardia lamblia* cysts, viruses, or other pathogenic organisms of public health concern to comply with the treatment technique requirements of Part 6 of this chapter.

((~~(192)~~)) (196) "Primary standards" means standards based on chronic, nonacute, or acute human health effects.

((~~(193)~~)) (197) "Primary turbidity standard" means an accurately prepared formazin solution or commercially prepared polymer solution of known turbidity (prepared in accordance with "standard methods") that is used to calibrate bench model and continuous turbidimeters (instruments used to measure turbidity).

((~~(194)~~)) (198) "Project approval application (PAA)" means a department form documenting ownership of water system, design engineer for the project, and type of project.

((~~(195)~~)) (199) "Protected groundwater source" means a groundwater source the purveyor shows to the department's satisfaction as protected from potential sources of contamination on the basis of hydrogeologic data and/or satisfactory water quality history.

((~~(196)~~)) (200) "psi" means pounds per square inch.

((~~(197)~~)) (201) "Public forum" means a meeting open to the general public that allows for their participation.

((~~(198)~~)) (202) "Public water system" is defined and referenced under WAC 246-290-020.

((~~(199)~~)) (203) "Purchased source" means water a purveyor purchases from a public water system not under the control of the purveyor for distribution to the purveyor's consumers.

((~~(200)~~)) (204) "Purveyor" means an agency, subdivision of the state, municipal corporation, firm, company, mutual or cooperative association, institution, partnership, or person or other entity owning or operating a public water system. Purveyor also means the authorized agents of these entities.

((~~(201)~~)) (205) "PVBA" means a pressure vacuum breaker assembly.

((~~(202)~~)) (206) "Reclaimed water" means effluent derived in any part from sewage from a wastewater treatment system that has been adequately and reliably treated, so that as a result of that treatment,

it is suitable for beneficial use or a controlled use that would not otherwise occur, and it is no longer considered wastewater.

((~~(203)~~)) (207) "Record drawings" means the drawings bearing the seal and signature of a professional engineer that reflect the modifications made to construction documents, documenting actual constructed conditions of the water system facilities.

((~~(204)~~)) (208) "Recreational tract" means an area that is clearly defined for each occupant, but has no permanent structures with internal plumbing, and the area has been declared in the covenants or on the recorded plat in order to be eligible for reduced design considerations.

((~~(205)~~)) (209) "Regional public water supplier" means a water system that provides drinking water to one, or more, other public water systems.

((~~(206)~~)) (210) "Regularly" means four hours or more per day for four days or more per week.

((~~(207)~~)) (211) "Removal credit" means the level (expressed as a percent or log) of *Giardia* and virus removal the department grants a system's filtration process.

((~~(208)~~)) (212) "Repeat sample" means a sample collected to confirm the results of a previous analysis.

((~~(209)~~)) (213) "Resident" means an individual living in a dwelling unit served by a public water system.

((~~(210)~~)) (214) "Residual disinfectant concentration" means the analytical level of a disinfectant, measured in milligrams per liter, that remains in water following the application (dosing) of the disinfectant after some period of contact time.

((~~(211)~~)) (215) "Retail service area" means the specific area defined by the municipal water supplier where the municipal water supplier has a duty to provide service to all new service connections as set forth in RCW 43.20.260.

((~~(212)~~)) (216) "RPBA" means reduced pressure backflow assembly.

((~~(213)~~)) (217) "RPDA" means reduced pressure detector assembly.

((~~(214)~~)) (218) "Running annual average (RAA)" means the average of analytical results from compliance samples collected at the monitoring locations identified in WAC 246-290-300 during any consecutive four calendar quarters. If a system fails to collect the required number of samples, the RAA is based on the total number of samples collected. If a sample result is less than the SDRL, zero is used to calculate the RAA.

((~~(215)~~)) (219) "Same farm" means a parcel of land or series of parcels that are connected by covenants and devoted to the production of livestock or agricultural commodities for commercial purposes and does not qualify as a Group A public water system.

((~~(216)~~)) (220) "Sanitary defect" means a defect that could provide a pathway of entry for microbial contamination into the distribution system or that is indicative of a failure or imminent failure in a barrier that is already in place.

((~~(217)~~)) (221) "Sanitary survey" means a review, inspection, and assessment of a public water system, by the department or department designee, to determine the adequacy of the system and its operation for producing and distributing safe and reliable drinking water. Each survey includes, but is not limited to, an evaluation of the following components:

(a) Source;

(b) Treatment;

(c) Distribution system;

(d) Finished water storage;
(e) Pump, pump facilities, and controls;
(f) Monitoring, reporting, and data verification;
(g) System management and operation; and
(h) Operator compliance.

((~~(218)~~)) (222) "Satellite system management agency (SMA)" means a person or entity that is approved by the department to own or operate public water systems on a regional or county-wide basis without the necessity for a physical connection between the systems.

((~~(219)~~)) (223) "SCA" means a sanitary control area.

((~~(220)~~)) (224) "SDWA" means the Safe Drinking Water Act.

((~~(221)~~)) (225) "Seasonal source" means a public water system source used on a regular basis, that is not a permanent or emergency source.

((~~(222)~~)) (226) "Seasonal system" means a noncommunity water system defined in WAC 246-290-020 that is not operated as a public water system on a year-round basis and starts up and shuts down at the beginning and end of each operating season.

((~~(223)~~)) (227) "Secondary standards" means standards based on factors other than health effects.

((~~(224)~~)) (228) "SEPA" means the State Environmental Policy Act.

((~~(225)~~)) (229) "Service area" means the specific area a water system currently serves and areas where future water service is planned. A wholesale system may include areas where it provides wholesale water to other public water systems in its service area. A water system in a CWSSA includes its future service area in its service area as "future service area" as defined under chapters 70A.100 RCW and 246-293 WAC.

((~~(226)~~)) (230) "Service connection" means a connection to a public water system designed to provide potable water to a single family residence, or other residential or nonresidential population. When the connection provides water to a residential population without clearly defined single family residences, the following formulas are used in determining the number of services to be included as residential connections on the WFI form:

(a) Divide the average population served each day by two and one-half; or

(b) Using actual water use data, calculate the total ERUs represented by the service connection in accordance with department design guidance.

(c) The calculated number of services is never less than one.

((~~(227)~~)) (231) "Severe health cross-connection hazard" means a cross-connection which could impair the quality of potable water and create an immediate, severe public health hazard through poisoning or spread of disease by contaminants from radioactive material processing plants, nuclear reactors, or wastewater treatment plants.

((~~(228)~~)) (232) "Simple disinfection" means any form of disinfection that requires minimal operational control in order to maintain the disinfection at proper functional levels, and that does not pose safety concerns that would require special care, equipment, or expertise. Examples include hypochlorination, UV-light, contactor chlorination, or any other form of disinfection practice that is safe to use and easy to routinely operate and maintain.

((~~(229)~~)) (233) "Slow sand filtration" means a process involving passage of source water through a bed of sand at low velocity (generally less than 0.10 gpm/ft^2) that results in substantial particulate

removal (> 2-log *Giardia lamblia* cysts) by physical and biological mechanisms.

((~~(230)~~)) (234) "Societal perspective" means:

A point of view that includes a broad spectrum of public benefits including, but not limited to:

(a) Enhanced system reliability;

(b) Savings that result from delaying, deferring, or minimizing capital costs; and

(c) Environmental benefits such as increased water in streams, improvements in aquifer recharge and other environmental factors.

((~~(231)~~)) (235) "Source meter" means a meter that measures total output of a water source over specific time periods.

((~~(232)~~)) (236) "Source water" means untreated water that is not subject to recontamination by surface runoff and:

(a) For unfiltered systems, enters the system immediately before the first point of disinfectant application; and

(b) For filtered systems, enters immediately before the first treatment unit of a water treatment facility.

((~~(233)~~)) (237) "Special purpose investigation (SPI)" means on-site inspection of a public water system by the department or designee to address a potential public health concern, regulatory violation, or consumer complaint.

((~~(234)~~)) (238) "Special purpose sample" means a sample collected for reasons other than the monitoring compliance specified in this chapter.

((~~(235)~~)) (239) "Spring" means a source of water where an aquifer comes in contact with the ground surface.

((~~(236)~~)) (240) "Standard methods" means the book, titled *Standard Methods for the Examination of Water and Waste Water,* jointly published by the American Public Health Association, American Water Works Association (AWWA), and Water Pollution Control Federation. This book is available through public libraries or may be ordered from AWWA, 6666 West Quincy Avenue, Denver, Colorado 80235. The edition to be used is that specified by EPA for the relevant drinking water parameter in 40 C.F.R. Part 141.

((~~(237)~~)) (241) "Standby storage" means the volume of stored water available for use during a loss of source capacity, power, or similar short-term emergency.

((~~(238)~~)) (242) "State action level (SAL)" means the concentration of a contaminant or group of contaminants, without an MCL, established to protect public health in accordance with WAC 246-290-315 and which, if exceeded, triggers actions a purveyor takes in accordance with WAC 246-290-320.

((~~(239)~~)) (243) "State board of health" and "board" means the board created by RCW 43.20.030.

((~~(240)~~)) (244) "State building code" means the codes adopted by and referenced in chapter 19.27 RCW; the state energy code; and any other codes so designated by the Washington state legislature as adopted and amended by the council.

((~~(241)~~)) (245) "State detection reporting limit (SDRL)" means the minimum reportable detection of a contaminant established in WAC 246-390-075.

((~~(242)~~)) (246) "State revolving fund (SRF)" means the revolving loan program financed by the state and federal governments and managed by the state for the purpose of assisting water systems to meet their capital needs associated with complying with the federal Safe Drinking Water Act under chapter 246-296 WAC.

((~~(243)~~)) (247) "State significant noncomplier (SSNC)" means a system that is violating or has violated department rules, and the violations may create, or have created an imminent or a significant risk to human health.

The violations include, but are not limited to:

(a) Repeated violations of monitoring requirements;

(b) Failure to address an exceedance of permissible levels of regulated contaminants;

(c) Failure to comply with treatment technique standards or requirements;

(d) Failure to comply with waterworks operator certification requirements; or

(e) Failure to submit to a sanitary survey.

((~~(244)~~)) (248) "Subpart H System" see definition for "surface water system."

((~~(245)~~)) (249) "Surface water" means a body of water open to the atmosphere and subject to surface runoff.

((~~(246)~~)) (250) "Surface water system" means a public water system that uses in whole, or in part, source water from a surface supply, or GWI supply. This includes systems that operate surface water treatment facilities, and systems that purchase "completely treated water". A "surface water system" is also referred to as a "Subpart H System" in some federal regulatory language adopted by reference and the two terms are considered equivalent for the purposes of this chapter.

((~~(247)~~)) (251) "Susceptibility assessment" means the completed susceptibility assessment survey form for groundwater sources, or a surface water checklist for surface water sources, developed by the department to evaluate the hydrologic setting of the water source and assess its contribution to the source's overall susceptibility to contamination from surface activities.

((~~(248)~~)) (252) "SUVA" means specific ultraviolet absorption.

((~~(249)~~)) (253) "SVBA" means spill resistant vacuum breaker assembly.

((~~(250)~~)) (254) "SWTR" means the surface water treatment rule.

((~~(251)~~)) (255) "Synthetic organic chemical (SOC)" means a manufactured carbon-based chemical.

((~~(252)~~)) (256) "System capacity" means the system's operational, technical, managerial, and financial capability to achieve and maintain compliance with all relevant local, state, and federal plans and regulations.

((~~(253)~~)) (257) "System physical capacity" means the maximum number of service connections or equivalent residential units (ERUs) that the system can serve when considering the limitation of each system component such as source, treatment, storage, transmission, or distribution, individually and in combination with each other.

((~~(254)~~)) (258) "T" means disinfectant contact time in minutes.

((~~(255)~~)) (259) "Time-of-travel" means the time required for groundwater to move through the water bearing zone from a specific point to a well.

((~~(256)~~)) (260) "TNC" means transient noncommunity.

((~~(257)~~)) (261) "TOC" means total organic carbon.

((~~(258)~~)) (262) "Too numerous to count (TNTC)" means the total number of bacterial colonies exceeds 200 on a 47-mm diameter membrane filter used for coliform detection.

((~~(259)~~)) (263) "Tracer study" means a field study conducted to determine the disinfectant contact time, T, provided by a water system

component, such as a clearwell or storage reservoir, used for *Giardia lamblia* cyst and virus inactivation. The study involves introducing a tracer chemical at the inlet of the contact basin and measuring the resulting outlet tracer concentration as a function of time.

((~~(260)~~)) (264) "Transmission line" means pipes used to convey water from source, storage, or treatment facilities to points of distribution or distribution mains, and from source facilities to treatment or storage facilities. This also can include transmission mains connecting one section of distribution system to another section of distribution system as long as this transmission main is clearly defined on the plans and no service connections are allowed along the transmission main.

((~~(261)~~)) (265) "Treatment technique (TT) requirement" means a department-established requirement for a public water system to provide treatment, such as filtration or disinfection, as defined by specific design, operating, and monitoring requirements. A TT requirement is established in lieu of a primary MCL when monitoring for the contaminant is not economically or technologically feasible.

((~~(262)~~)) (266) "Triggered source water monitoring" means collection of groundwater source samples as a result of a total coliform-positive routine sample in the distribution system under WAC 246-290-300(3).

((~~(263)~~)) (267) "Trihalomethane (THM)" means one of a family of organic compounds, named as derivatives of methane, where three of the four hydrogen atoms in methane are each substituted by a halogen atom in the molecular structure. THMs may occur when chlorine, a halogen, is added to water containing organic material and are generally found in water samples as disinfection byproducts.

((~~(264)~~)) (268) "TTHM" means total trihalomethane.

((~~(265)~~)) (269) "Turbidity event" means a single day or series of consecutive days, not to exceed ((~~fourteen~~)) 14, when one or more turbidity measurement each day exceeds 5 NTU.

((~~(266)~~)) (270) "Two-stage lime softening" means a process in which chemical addition and hardness precipitation occur in each of two distinct unit clarification processes in series prior to filtration.

((~~(267)~~)) (271) "T10" means the time it takes ((~~ten~~)) 10 percent of the water passing through a system contact tank intended for use in the inactivation of *Giardia lamblia* cysts, viruses, and other microorganisms of public health concern, as determined from a tracer study conducted at peak hourly flow or from published engineering reports or guidance documents for similarly configured tanks.

((~~(268)~~)) (272) "ug/L" means micrograms per liter.

((~~(269)~~)) (273) "UL" means the Underwriters Laboratories, Inc.

((~~(270)~~)) (274) "umhos/cm" means micromhos per centimeter.

((~~(271)~~)) (275) "Unapproved auxiliary water supply" means a water supply (other than the purveyor's water supply) on or available to the consumer's premises that is either not approved for human consumption by the health agency having jurisdiction or is not otherwise acceptable to the purveyor.

((~~(272)~~)) (276) "Uncovered finished water storage facility" means a tank, reservoir, or other facility used to store water, which will undergo no further treatment to reduce microbial pathogens except residual disinfection and is directly open to the atmosphere without a suitable water-tight roof or cover.

((~~(273)~~)) (277) "Uniform Plumbing Code (UPC)" means the code adopted under RCW 19.27.031(4) and implemented under chapter 51-56

WAC. This code establishes statewide minimum plumbing standards applicable within the property lines of the consumer's premises.

((~~(274)~~)) (278) "Used water" means water which has left the control of the purveyor.

((~~(275)~~)) (279) "UTC" means the utilities and transportation commission.

((~~(276)~~)) (280) "Verification" means to demonstrate the results of a sample to be precise by analyzing a duplicate sample. Verification occurs when analysis results fall within plus or minus ((~~thirty~~)) 30 percent of the original sample.

((~~(277)~~)) (281) "Virus" means a virus of fecal origin which is infectious to humans and transmitted through water.

((~~(278)~~)) (282) "Volatile organic chemical (VOC)" means a manufactured carbon-based chemical that vaporizes quickly at standard pressure and temperature.

((~~(279)~~)) (283) "Voluntary curtailment" means a curtailment of water use requested, but not required of consumers.

((~~(280)~~)) (284) "WAC" means the Washington Administrative Code.

((~~(281)~~)) (285) "Waterborne disease outbreak" means the significant occurrence of acute infectious illness, epidemiologically associated with drinking water from a public water system, as determined by the appropriate local health agency or the department.

((~~(282)~~)) (286) "Water demand efficiency" means minimizing water use by the public water system's consumers through purveyor sponsored activities that may include, but are not limited to, distributing water saving devices, providing rebates or incentives to promote water efficient technologies or by providing water audits to homes, businesses, or landscapes.

((~~(283)~~)) (287) "Water facilities inventory (WFI) form" means the department form summarizing each public water system's characteristics.

((~~(284)~~)) (288) "Water right" means a certificated water right, water right permit, valid claim, or other authorization, on record with or accepted by the department of ecology, authorizing the beneficial use of water in accordance with all applicable state laws.

((~~(285)~~)) (289) "Water right self-assessment" means an evaluation of the legal ability of a water system to use water for existing or proposed usages in conformance with state water right laws. The assessment may be done by a water system, a purveyor, the department of ecology, or any combination thereof.

((~~(286)~~)) (290) "Watershed" means the region or area that:

(a) Ultimately drains into a surface water source diverted for drinking water supply; and

(b) Affects the physical, chemical, microbiological, and radiological quality of the source.

((~~(287)~~)) (291) "Water shortage" means a situation during which the water supplies of a system cannot meet normal water demands for the system, including peak periods.

((~~(288)~~)) (292) "Water shortage response plan" means a plan outlining policies and activities to be implemented to reduce water use on a short-term basis during or in anticipation of a water shortage.

((~~(289)~~)) (293) "Water supply characteristics" means the factors related to a public water system's source of water supply that may affect its availability and suitability to provide for both short-term and long-term needs.

Factors include, but are not limited to:

(a) Source location;

(b) Name of any body of water and water resource inventory area from which water is diverted or withdrawn;

(c) Production capacity;

(d) The source's natural variability;

(e) The system's water rights for the source;

(f) Other legal demands on the source such as water rights for other uses;

(g) Conditions established to protect species listed under the Endangered Species Act in 50 C.F.R. 17.11;

(h) Instream flow restrictions established under Title 173 WAC; and

(i) Any conditions established by watershed plans approved under chapter 90.82 RCW and RCW 90.54.040(1) or salmon recovery plans under chapter 77.85 RCW.

((~~(290)~~)) (294) "Water supply efficiency" means increasing a public water system's transmission, storage and delivery potential through activities that may include, but are not limited to:

(a) System-wide water audits;

(b) Documenting authorized uses;

(c) Conducting leak surveys; and

(d) Repairs on:

(i) Meters;

(ii) Lines;

(iii) Storage facilities; and

(iv) Valves.

((~~(291)~~)) (295) "Water use efficiency (WUE)" means increasing water supply efficiency and water demand efficiency to minimize water withdrawals and water use.

((~~(292)~~)) (296) "Water use efficiency program" means policies and activities focusing on increasing water supply efficiency and water demand efficiency to minimize water withdrawals and water use.

((~~(293)~~)) (297) "Well field" means a group of wells one purveyor owns or controls that:

(a) Draw from the same aquifer or aquifers as determined by comparable inorganic chemical analysis and comparable static water level and top of the open interval elevations; and

(b) Discharge water through a common pipe and the common pipe shall allow for collection of a single sample before the first distribution system connection.

((~~(294)~~)) (298) "Wellhead protection area (WHPA)" means the portion of a well's, wellfield's or spring's zone of contribution defined using WHPA criteria established by the department.

((~~(295)~~)) (299) "Wholesale system" means a public water system that treats source water as necessary to produce finished water and then delivers some or all of that finished water to another public water system. Delivery may be through a direct connection or through the distribution system of one or more consecutive systems.

((~~(296)~~)) (300) "Zone of contribution" means the area surrounding a pumping well or spring that encompasses all areas or features that supply groundwater recharge to the well or spring.

AMENDATORY SECTION (Amending WSR 17-01-062, filed 12/14/16, effective 1/14/17)

WAC 246-290-025 Adoption by reference. The following sections and subsections of Title 40 Code of Federal Regulations (C.F.R.) Part 141 National Primary Drinking Water Regulations and Part 143 National Secondary Drinking Water Regulations revised as of ((~~July 1, 2016~~)) June 25, 2024, are adopted by reference:

((~~141.2 Definitions. Only those definitions listed as follows:~~

~~Action level;~~

~~Corrosion inhibitor;~~

~~Effective corrosion inhibitor residual;~~

~~Enhanced coagulation;~~

~~Enhanced softening;~~

~~First draw sample;~~

~~Haloacetic acids (five) (HAA5);~~

~~Large water system;~~

~~Lead service line;~~

~~Maximum residual disinfectant level (MRDL);~~

~~Maximum residual disinfectant level goal (MRDLG);~~

~~Medium-size water system;~~

~~Optimal corrosion control treatment;~~

~~Service line sample;~~

~~Single family structure;~~

~~Small water system;~~

~~Specific ultraviolet absorption (SUVA); and~~

~~Total Organic Carbon (TOC).~~

~~141.13~~	~~Maximum contaminant levels for turbidity.~~
~~141.22~~	~~Turbidity sampling and analytical requirements.~~
~~141.23(a) - excluding (i)(2)~~	~~141.23(j), Inorganic chemical sampling.~~
~~141.23(m) -~~	~~141.23(o)~~
~~141.24(a) -~~	~~141.24(d), Organic chemicals, sampling and analytical requirements.~~

~~141.24 (f)(1) - 141.24 (f)(15),~~

~~141.24 (f)(18), 141.24 (f)(19),~~

~~141.24 (f)(21), 141.24 (f)(22)~~

~~141.24 (g)(1) - 141.24 (g)(9),~~

~~141.24 (g)(12) - 141.24 (g)(14),~~

~~141.24 (h)(1) - 141.24 (h)(11),~~

~~141.24 (h)(14) - 141.24 (h)(17)~~

~~141.24 (h)(20)~~

~~141.25(a),~~	~~141.25 (c) - (d), Analytical methods for radioactivity.~~
~~141.26~~	~~Monitoring frequency and compliance for radionuclides in community water systems.~~
~~141.31(d)~~	~~Reporting requirements.~~
~~141.33(e)~~	~~Record maintenance.~~

~~141.40~~	~~Monitoring requirements for unregulated contaminants.~~
~~141.61~~	~~Maximum contaminant levels for organic contaminants.~~
~~141.62, excluding (b)~~	~~Maximum contaminant levels for inorganic contaminants.~~
~~141.63(e)~~	~~Maximum contaminant levels (MCLs) for microbiological contaminants.~~
~~141.64~~	~~Maximum contaminant levels for disinfection byproducts.~~
~~141.65(c)~~	~~Maximum Residual Disinfectant Levels.~~
~~141.66~~	~~Maximum contaminant levels for radionuclides.~~
~~Control of Lead and Copper~~	
~~141.80, excluding (c)(3)(v)~~	~~General requirements.~~
~~141.81~~	~~Applicability of corrosion control treatment steps to small, medium-size and large water systems.~~
~~141.82(a) -~~	~~141.82(h) Description of corrosion control treatment requirements.~~
~~141.83~~	~~Source water treatment requirements.~~
~~141.84~~	~~Lead service line replacement requirements.~~
~~141.85~~	~~Public education and supplemental monitoring requirements.~~
~~141.86 (a) - (f)~~	~~Monitoring requirements for lead and copper in tap water.~~
~~141.87~~	~~Monitoring requirements for water quality parameters.~~
~~141.88~~	~~Monitoring requirements for lead and copper in source water.~~
~~141.89~~	~~Analytical methods.~~
~~141.90, excluding (a)(4)~~	~~Reporting requirements.~~
~~141.91~~	~~Recordkeeping requirements.~~
~~Disinfectants and Disinfection Byproducts (D/DBP)~~	
~~141.130~~	~~General requirements.~~
~~141.131~~	~~Analytical requirements.~~
~~141.132, excluding (c)(1)(i)~~	~~Monitoring requirements.~~
~~141.133~~	~~Compliance requirements.~~
~~141.134~~	~~Reporting and recordkeeping requirements.~~
~~141.135~~	~~Treatment technique for control of disinfection byproduct precursors.~~
~~Subpart O - Consumer Confidence Reports~~	
~~141.153 (h)(6) and (7)~~	~~Contents of the reports.~~
~~Enhanced Filtration and Disinfection - Systems Serving 10,000 or More People~~	
~~141.175(b)~~	~~Reporting and recordkeeping requirements.~~
~~Subpart Q - Public Notification of Drinking Water Violations~~	

~~141.201, excluding (3)(ii) of Table 1~~	~~General public notification requirements.~~
~~141.202, excluding (3) of Table 1~~	~~Tier 1 Public Notice - Form, manner, and frequency of notice.~~
~~141.203~~	~~Tier 2 Public Notice - Form, manner, and frequency of notice.~~
~~141.204~~	~~Tier 3 Public Notice - Form, manner, and frequency of notice.~~
~~141.205~~	~~Content of the public notice.~~
~~141.206~~	~~Notice to new billing units or new customers.~~
~~141.207~~	~~Special notice of the availability of unregulated contaminant monitoring results.~~
~~141.208~~	~~Special notice for exceedances of the SMCL for fluoride.~~
~~141.211~~	~~Special notice for repeated failure to conduct monitoring of the source water for *Cryptosporidium* and for failure to determine bin classification or mean *Cryptosporidium* level.~~
~~Appendix A to Subpart Q of Part 141 - NPDWR violations and other situations requiring public notice~~	
~~Appendix B to Subpart Q of Part 141 - Standard health effects language for public notification~~	
~~Appendix C to Subpart Q of Part 141- List of acronyms used in public notification regulation~~	
~~141.400~~	~~General requirements and applicability.~~
~~141.402(c)~~	~~Groundwater source microbial monitoring and analytical methods.~~
~~141.403 (b)(3)(i) through (iii)~~	~~Treatment technique requirements for groundwater systems.~~
~~Subpart T - Enhanced Filtration and Disinfection - Systems Serving Fewer Than 10,000 People~~	
~~141.530 - 141.544~~	~~Disinfection profile and benchmark.~~
~~141.563~~	~~What follow-up action is my system required to take based on continuous turbidity monitoring?~~
~~141.570, excluding (c)~~	~~What does Subpart T require that my system report to the state?~~
~~Subpart U - Initial Distribution System Evaluations~~	
~~141.600 - 141.605~~	~~Initial distribution system evaluations.~~
~~Subpart V - Stage 2 Disinfection Byproducts Requirements~~	
~~141.620 - 141.629, excluding 624~~	~~Stage 2 Disinfection Byproducts Requirements.~~
~~Subpart W - Enhanced Treatment for *Cryptosporidium*~~	
~~141.700-722 Enhanced Treatment for *Cryptosporidium*~~	
~~Subpart Y - Revised Total Coliform Rule~~	
~~141.852~~	~~Analytical methods and laboratory certification.~~
~~141.860 (c) - (d)~~	~~Violations~~

~~Part 143 - National Secondary Drinking Water Regulations~~

~~143.1 Purpose.~~

~~143.2 Definitions.~~

~~143.3 Secondary maximum contaminant levels.~~

~~143.4 Monitoring.~~))

141.2	Definitions. Only those definitions listed as follows:
	Action level;
	Corrosion inhibitor;
	Effective corrosion inhibitor residual;
	Enhanced coagulation;
	Enhanced softening;
	First draw sample;
	Haloacetic acids (five) (HAA5);
	Large water system;
	Lead service line;
	Maximum residual disinfectant level (MRDL);
	Maximum residual disinfectant level goal (MRDLG);
	Medium-size water system;
	Optimal corrosion control treatment;
	Single family structure;
	Small water system;
	Specific ultraviolet absorption (SUVA); and
	Total Organic Carbon (TOC).
141.13	Maximum contaminant levels for turbidity.
141.22	Turbidity sampling and analytical requirements.
141.23(a) through (j) and (m) through (o), excluding (i)(2), (k), and (l)	Inorganic chemical sampling and compliance.
141.24(f)(1) through (15), (18), (19), (21), and (22)	Volatile organic monitoring and compliance.
141.24(h), excluding (13), (18), and (19)	Synthetic organic monitoring and compliance, except PFAS.
141.25(a), (c), and (d)	Analytical methods for radioactivity.
141.26	Monitoring frequency and compliance for radionuclides in community water systems.
141.31(d)	Reporting requirements.
141.33(e)	Record maintenance.
141.40	Monitoring requirements for unregulated contaminants.
141.61	Maximum contaminant levels for organic contaminants.
141.62, excluding (b)	Maximum contaminant levels for inorganic contaminants.
141.63(e)	Maximum contaminant levels (MCLs) for microbiological contaminants.
141.64	Maximum contaminant levels for disinfection byproducts.
141.65(c)	Maximum Residual Disinfectant Levels.
141.66	Maximum contaminant levels for radionuclides.
Control of Lead and Copper	
141.80, excluding (c)(3)(v)	General requirements.
141.81	Applicability of corrosion control treatment steps to small, medium-size and large water systems.
141.82(a) through (h)	Description of corrosion control treatment requirements.
141.83	Source water treatment requirements.

141.84	Lead service line replacement requirements.
141.85	Public education and supplemental monitoring requirements.
141.86(a) through (f)	Monitoring requirements for lead and copper in tap water.
141.87	Monitoring requirements for water quality parameters.
141.88	Monitoring requirements for lead and copper in source water.
141.89	Analytical methods.
141.90, excluding (a)(4)	Reporting requirements.
141.91	Recordkeeping requirements.
Disinfectants and Disinfection Byproducts (D/DBP)	
141.130	General requirements.
141.131	Analytical requirements.
141.132, excluding (c)(1)(i)	Monitoring requirements.
141.133	Compliance requirements.
141.134	Reporting and recordkeeping requirements.
141.135	Treatment technique for control of disinfection byproduct precursors.
Subpart O - Consumer Confidence Reports	
141.153 (h)(6) and (7)	Contents of the reports.
Enhanced Filtration and Disinfection - Systems Serving 10,000 or More People	
141.175(b)	Reporting and recordkeeping requirements.
Subpart Q - Public Notification of Drinking Water Violations	
141.201, excluding (3)(ii) of Table 1	General public notification requirements.
141.202, excluding (3) of Table 1	Tier 1 Public Notice - Form, manner, and frequency of notice.
141.203	Tier 2 Public Notice - Form, manner, and frequency of notice.
141.204	Tier 3 Public Notice - Form, manner, and frequency of notice.
141.205	Content of the public notice.
141.206	Notice to new billing units or new customers.
141.207	Special notice of the availability of unregulated contaminant monitoring results.
141.208	Special notice for exceedances of the SMCL for fluoride.
141.211	Special notice for repeated failure to conduct monitoring of the source water for *Cryptosporidium* and for failure to determine bin classification or mean *Cryptosporidium* level.
Appendix A to Subpart Q of Part 141	NPDWR violations and other situations requiring public notice.
Appendix B to Subpart Q of Part 141	Standard health effects language for public notification.
Appendix C to Subpart Q of Part 141	List of acronyms used in public notification regulation.
141.400	General requirements and applicability.
141.402(c)	Groundwater source microbial monitoring and analytical methods.
141.403 (b)(3)(i) through (iii)	Treatment technique requirements for groundwater systems.
Subpart T - Enhanced Filtration and Disinfection - Systems Serving Fewer Than 10,000 People	
141.530 through 141.544	Disinfection profile and benchmark.
141.563	What follow-up action is my system required to take based on continuous turbidity monitoring?
141.570, excluding (c)	What does Subpart T require that my system report to the state?
Subpart U - Initial Distribution System Evaluations	
141.600 through 141.605	Initial distribution system evaluations.
Subpart V - Stage 2 Disinfection Byproducts Requirements	
141.620 through 141.629, excluding 141.624	Stage 2 Disinfection Byproducts Requirements.

Subpart W - Enhanced Treatment for *Cryptosporidium*	
141.700 through 141.722	Enhanced Treatment for *Cryptosporidium.*
Subpart Y - Revised Total Coliform Rule	
141.852	Analytical methods and laboratory certification.
141.860(c) and (d)	Violations.
141.904	Reporting and Recording Keeping Requirements.
141.905	Violations.
Part 143 - National Secondary Drinking Water Regulations	
143.1	Purpose.
143.2	Definitions.
143.3	Secondary maximum contaminant levels.
143.4	Monitoring.

Copies of the incorporated sections and subsections of Title 40 C.F.R. are available from the Department of Health online at: http://www/doh.wa.gov/CommunityandEnvironment/DrinkingWater/RegulationandCompliance/Rules, or P.O. Box 47822, Olympia, Washington 98504-7822, or by calling the department's drinking water hotline at 800-521-0323.

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-300 Monitoring requirements. (1) General.

(a) The monitoring requirements specified in this section are minimums. The department may require additional monitoring when:

(i) Contamination is present or suspected in the water system;

(ii) A groundwater source is determined to be a potential GWI;

(iii) The degree of source protection is not satisfactory;

(iv) Additional monitoring is needed to verify source vulnerability for a requested monitoring waiver;

(v) Under other circumstances as identified in a department order; or

(vi) Additional monitoring is needed to evaluate continuing effectiveness of a treatment process where problems with the treatment process may exist.

(b) Special purpose samples collected by the purveyor do not count toward fulfillment of the monitoring requirements of this chapter unless the quality of data and method of sampling and analysis are acceptable to the department.

(c) The purveyor shall ensure samples required by this chapter are collected, transported, and submitted for analysis according to EPA-approved methods. The analyses must be performed by a laboratory accredited by the state using EPA-approved methods or other department-approved methods. Qualified water utility, accredited laboratory, health department personnel, and other parties approved by the department may conduct measurements for pH, temperature, residual disinfectant concentration, alkalinity, bromide, chlorite, TOC, SUVA, turbidity, calcium, conductivity, orthophosphate, and silica as required by this chapter, provided, these measurements are made according to EPA approved methods.

(d) Compliance samples required by this chapter must be taken at locations listed in Table 4 of this section.

(e) Purveyors failing to comply with a monitoring requirement shall notify:

(i) The department under WAC 246-290-480; and

(ii) The owner or operator of any consecutive system served and the appropriate water system users under 40 C.F.R. 141.201 and Part 7, Subpart A of this chapter.

(2) Selling and receiving water.

(a) Source monitoring. Purveyors, with the exception of those that "wheel" water to their consumers (i.e., sell water that has passed through another purchasing purveyor's distribution system), shall conduct source monitoring under this chapter for the sources under their control. The level of monitoring must satisfy the monitoring requirements associated with the total population served by the source.

(b) Distribution system monitoring. The purveyor of a system that receives and distributes water shall perform distribution-related monitoring requirements. Monitoring must include, but not be limited to, the following:

(i) Collect coliform samples under subsection (3) of this section;

(ii) Collect disinfection byproduct samples as required by subsection (6) of this section;

(iii) Perform the distribution system residual disinfectant concentration monitoring under subsection (6) of this section, and as required under WAC 246-290-451, 246-290-664, or 246-290-694. Systems with fewer than ((~~one hundred~~)) 100 connections shall measure residual disinfectant concentration at the same time and location that a routine or repeat coliform sample is collected, unless the department determines that more frequent monitoring is necessary to protect public health;

(iv) Perform lead and copper monitoring required under 40 C.F.R. 141.86, 141.87, and 141.88;

(v) Perform the distribution system monitoring under 40 C.F.R. 141.23(b) for asbestos if applicable;

(vi) Other monitoring as required by the department.

(c) Reduced monitoring for regional programs. The receiving purveyor may receive reductions in the coliform, lead and copper, disinfection byproduct (including TTHMs and HAA5) and distribution system disinfectant residual concentration monitoring requirements, provided the receiving system:

(i) Purchases water from a purveyor that has a department-approved regional monitoring program;

(ii) Has a written agreement with the supplying system or regional water supplier that is acceptable to the department, and which identifies the responsibilities of both the supplying and receiving system(s) with regards to monitoring, reporting and maintenance of the distribution system; and

(iii) Has at least one compliance monitoring location for disinfection byproducts, if applicable.

(d) Periodic review of regional programs. The department may periodically review the sampling records of public water systems participating in a department-approved monitoring program to determine if continued reduced monitoring is appropriate. If the department determines a change in the monitoring requirements of the receiving system is appropriate:

(i) The department shall notify the purveyor of the change in monitoring requirements; and

(ii) The purveyor shall conduct monitoring as directed by the department.

(3) Bacteriological.

(a) The purveyor shall be responsible for collection and submittal of coliform samples from representative points throughout the distribution system. Samples must be collected after the first service and at regular time intervals each month the system provides water to consumers. Samples must be collected that represent normal system operating conditions.

(i) Systems providing disinfection treatment shall measure the residual disinfectant concentration within the distribution system at the same time and location of routine and repeat samples.

(ii) Systems providing disinfection treatment shall assure that disinfectant residual concentrations are measured and recorded on all coliform sample report forms submitted for compliance purposes.

(b) Coliform monitoring plan.

(i) Systems shall develop a written coliform monitoring plan that identifies sampling sites and a sample collection schedule that are representative of water throughout the distribution system. The plan is subject to department review and approval. Systems shall collect total coliform samples according to the plan. Monitoring may take place at a customer's premises, dedicated sampling station, or other designated compliance sampling location. Routine and repeat sample sites and any sampling points necessary to meet the requirements of Part 6 of this chapter and ((~~WAC 246-290-300~~)) subsection (3)(h) of this section must be identified in the plan.

(ii) Systems shall collect samples at regular time intervals throughout the month, except for systems that use groundwater and serve ((~~four thousand nine hundred~~)) 4,900 or fewer people may collect all required samples on a single day if the samples are taken from different sites.

(iii) Systems shall take at least the minimum number of required samples even if the system has had an *E. coli* MCL violation or has exceeded the coliform treatment technique triggers in WAC 246-290-320(2).

(iv) Systems may conduct more compliance monitoring than is required under subsection (3)(b) of this section to investigate potential problems in the distribution system and use monitoring as a tool to assist in identifying problems. Systems may take more than the minimum number of required routine samples and must include the results in calculating whether or not the coliform treatment technique triggers in WAC 246-290-320(2) have been exceeded only if the samples are taken in accordance with the plan and are representative of water throughout the distribution system.

(v) Systems shall identify repeat monitoring locations in the plan. Unless the provisions of subsection (3)(b)(i) through (iv) of this section are met, the system shall collect at least one repeat sample from the sample tap where the original total coliform-positive sample was taken, and at least one repeat sample at a tap within five service connections upstream and at least one repeat sample at a tap within five service connections downstream of the original sample site. If a total coliform-positive sample is at the end of the distribution system, or one service connection away from the end of the distribution system, the system shall still take all required repeat samples. The department may allow an alternative sampling location in

lieu of the requirement to collect at least one repeat sample upstream or downstream of the original sampling site. Systems may propose repeat monitoring locations to the department that the system believes to be representative of a pathway for contamination of the distribution system. A system may elect to specify either alternative fixed locations or criteria for selecting repeat sampling sites on a situational basis in a standard operating procedure (SOP) in its plan. The system shall design its SOP to focus the repeat samples at locations that best verify and determine the extent of potential contamination of the distribution system area based on specific situations. The department may modify the SOP or require alternative monitoring locations as needed.

(vi) The purveyor shall:

(A) Keep the coliform monitoring plan on file with the system and make it available to the department for inspection upon request;

(B) Revise or expand the plan at any time the plan no longer ensures representative monitoring of the system, or as directed by the department; and

(C) Submit the plan to the department for review and approval when requested and as part of the water system plan required under WAC 246-290-100.

(c) Special purpose coliform samples. Special purpose coliform samples, such as those taken to determine whether disinfection practices are sufficient following pipe placement, replacement, or repair, must not be used to determine whether or not the coliform treatment technique trigger has been exceeded. Repeat samples taken in accordance with subsection (3) of this section are not considered special purpose coliform samples, and must be used to determine whether or not the coliform treatment technique trigger has been exceeded.

(d) Invalidation of total coliform samples. A total coliform-positive sample invalidated under subsection (3) of this section does not count toward meeting the minimum monitoring requirements of this section.

(i) The department may invalidate a total coliform-positive sample if one or more of the following conditions are met:

(A) The laboratory establishes that improper sample analysis caused the total coliform-positive result;

(B) The department, on the basis of the results of repeat samples collected as required under subsection (3) of this section, determines that the total coliform-positive samples resulted from a domestic or other nondistribution system plumbing problem. The department may not invalidate a sample on the basis of repeat sample results unless all repeat samples collected at the same tap as the original total coliform-positive sample are also total coliform-positive, and all repeat samples collected at a location other than the original tap are total coliform-negative. For example, the department may not invalidate a total coliform-positive sample on the basis of repeat samples if all the repeat samples are total coliform-negative, or if the system has only one service connection; or

(C) The department has substantial grounds to believe that a total coliform-positive result is due to a circumstance or condition that does not reflect water quality in the distribution system. In this case, the system shall still collect all repeat samples required under subsection (3) of this section, and use the samples to determine whether a coliform treatment technique trigger under WAC 246-290-320(2) has been exceeded.

(ii) Unless total coliforms are detected, a laboratory shall invalidate a total coliform sample if the sample produces a turbid culture in the absence of gas production using an analytical method where gas formation is examined such as the multiple-tube fermentation technique, produces a turbid culture in the absence of an acid reaction in the presence-absence coliform test, or exhibits confluent growth or produces colonies TNTC with an analytical method using a membrane filter such as a membrane filter technique. If a laboratory invalidates a sample because of such interference, the system shall collect another sample from the same location as the original sample within ((~~twenty-four~~)) 24 hours of notification of the interference problem, and have it analyzed for the presence of total coliforms. The system shall continue to resample within ((~~twenty-four~~)) 24 hours and have the samples analyzed until it obtains a valid result. The department may waive the ((~~twenty-four~~)) 24 hour time limit on a case-by-case basis.

(e) Monitoring frequency. The number of required routine coliform samples is based on total population served.

(i) Purveyors of community systems shall collect and submit for analysis no less than the number of routine samples listed in Table 2 of this section during each calendar month of operation;

(ii) Unless directed otherwise by the department, purveyors of noncommunity systems shall collect and submit for analysis no less than the number of samples required in Table 2 of this section. Each month's population must be based on the average daily population and must include all residents and nonresidents served during that month. During months when the average daily population served is less than ((~~twenty-five~~)) 25, routine sample collection is not required when:

(A) Using only protected groundwater sources;

(B) The system has a clean compliance history for a minimum of ((~~twelve~~)) 12 months;

(C) The system has no sanitary defects or significant deficiencies;

(D) The system has detected no total coliform-positive routine or repeat samples in the previous month; and

(E) The system has collected and submitted for analysis one routine sample during one of the previous two months.

(iii) Purveyors of NTNC and TNC systems are not required to collect routine samples in months when the population served is zero.

(iv) Purveyors of systems serving both a resident and a nonresident population shall base their minimum sampling requirement on the total of monthly populations served, both resident and nonresident as determined by the department, but no less than the minimum required in Table 2 of this section.

(v) Seasonal systems.

(A) In accordance with WAC 246-290-480 (2)(f)(ii), seasonal systems shall certify that a department-approved start-up procedure, which may include a requirement for start-up sampling, was completed prior to serving water to the public.

(B) Seasonal systems shall monitor every month that it is in operation unless it meets the criteria in subsection (3)(e)(ii) of this section.

(C) The department may exempt a seasonal system from some or all of the requirements in subsection (3)(e)(v)(A) of this section if the entire distribution system remains pressurized during the entire period that the system is not operating, except that systems that monitor less frequently than monthly shall still monitor during the vulnerable period designated by the department.

Table 2
Total Coliform Monitoring Frequency

Population served	Minimum number of samples per month
1 to 1,000*	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 12,900	10
12,901 to 17,200	15
17,201 to 21,500	20
21,501 to 25,000	25
25,001 to 33,000	30
33,001 to 41,000	40
41,001 to 50,000	50
50,001 to 59,000	60
59,001 to 70,000	70
70,001 to 83,000	80
83,001 to 96,000	90
96,001 to 130,000	100
130,001 to 220,000	120
220,001 to 320,000	150
320,001 to 450,000	180
450,001 to 600,000	210
600,001 to 780,000	240
780,001 to 970,000	270
970,001 to 1,230,000	300
1,230,001 to 1,520,000	330
1,520,001 to 1,850,000	360
1,850,001 to 2,270,000	390
2,270,001 to 3,020,000	420
3,020,001 to 3,960,000	450
3,960,001 or more	480

*Noncommunity systems using only protected groundwater sources and serving less than ((~~twenty-five~~)) 25 individuals, may collect and submit for analysis, one sample every three months per WAC 246-290-300 (3)(e)(ii).

(f) Repeat monitoring.

(i) If a routine sample taken under subsection (3) of this section is total coliform-positive, the system shall collect a set of repeat samples within ((~~twenty-four~~)) 24 hours of being notified of the positive result. Additional treatment, such as batch or shock chlorination must not be started prior to the collection of repeat samples

unless the department gives prior authorization. The purveyor shall contact the department to determine the best interim approach in this situation. The system shall collect no fewer than three repeat samples for each total coliform-positive sample found. The department may extend the ((~~twenty-four~~)) 24 hour limit on a case-by-case basis if the system has a logistical problem in collecting the repeat samples within ((~~twenty-four~~)) 24 hours that is beyond its control. Following the collection of repeat samples, and before the analytical results are known, the system may provide interim precautionary treatment or other means to protect public health.

(ii) The system shall collect all repeat samples on the same day, except the department may allow a system with a single connection to collect the required set of repeat samples over a three-day period or to collect a larger volume of repeat samples in one or more sample containers of any size, as long as the total volume collected is at least 300 mL.

(iii) The system shall collect an additional set of repeat samples in the manner specified in subsection (3)(f)(i) through (iii) of this section if one or more repeat samples in the current set of repeat samples is total coliform-positive. The system shall collect the additional set of repeat samples within ((~~twenty-four~~)) 24 hours of being notified of the positive result, unless the department extends the time limit as provided in subsection (3)(f)(i) of this section. The system shall continue to collect additional sets of repeat samples until either total coliforms are not detected in one complete set of repeat samples or the system determines that a coliform treatment technique trigger specified in WAC 246-290-320 (2)(a) has been exceeded as a result of a repeat sample being total coliform-positive and notifies the department. If a treatment technique trigger identified in WAC 246-290-320 (2)(a) is exceeded as a result of a routine sample being total coliform-positive, the system is required to conduct only one round of repeat monitoring for each total coliform-positive routine sample.

(iv) After a system collects a routine sample and before it gets the results of the analysis of that sample, if it collects subsequent routine samples from within five adjacent service connections of the initial sample, and the initial sample, after analysis, is found to contain total coliforms, then the system may count the subsequent samples as a repeat sample instead of as a routine sample.

(v) Results of all routine and repeat samples taken under subsection (3)(e) and (f) of this section not invalidated by the department under subsection (3)(d) of this section must be used to determine whether a coliform treatment technique trigger specified in WAC 246-290-320 (2)(a) has been exceeded.

(g) *E. coli* testing.

(i) If any routine or repeat sample is total coliform-positive, the system shall analyze that total coliform-positive culture medium to determine if *E. coli* are present. If *E. coli* are present, the system shall notify the department by the end of the day when the system is notified of the test result.

(ii) The department may allow a system, on a case-by-case basis, to forgo *E. coli* testing on a total coliform-positive sample if the system assumes that the total coliform-positive sample is *E. coli*-positive. Accordingly, the system shall notify the department as specified in WAC 246-290-320 (1)(a).

(h) Triggered source water monitoring.

(i) All groundwater systems with their own groundwater sources must conduct triggered source water monitoring unless the following conditions exist:

(A) The system has submitted a project report and received department approval that it provides at least 4-log treatment of viruses using inactivation, removal, or a department-approved combination of 4-log virus inactivation and removal before or at the first customer for each groundwater source; and

(B) The system is conducting compliance monitoring under WAC 246-290-453(2).

(ii) Any groundwater source sample required under this subsection (3) must be collected at the source prior to any treatment unless otherwise approved by the department.

(iii) Any groundwater source sample collected under this subsection (3) must be at least 100 mL in size and must be analyzed for *E. coli* using one of the analytical methods under 40 C.F.R. 141.402(c).

(iv) Groundwater systems shall collect at least one sample from each groundwater source in use at the time a routine sample collected under subsection (3) of this section is total coliform-positive and not invalidated under subsection (3)(d) of this section. These source samples must be collected within ((~~twenty-four~~)) 24 hours of notification of the total coliform-positive sample. The following exceptions apply:

(A) The ((~~twenty-four~~)) 24 hour time limit may be extended if granted by the department and will be determined on a case-by-case basis. If an extension is granted, the system shall sample by the deadline set by the department.

(B) Systems with more than one groundwater source may meet the requirements of subsection (3)(h)(iv) of this section by sampling a representative groundwater source or sources. The system shall have a department-approved triggered source water monitoring plan that identifies one or more groundwater sources that are representative of each monitoring site in the system's coliform monitoring plan under subsection (3)(b) of this section. The plan must be approved by the department before representative sampling will be allowed.

(v) Groundwater systems with an *E. coli* positive source water sample that is not invalidated under subsection (3)(h)(vii) of this section, shall:

(A) Notify the department by the end of the day when the system is notified of the test result.

(B) Provide Tier 1 public notice as required under Part 7, Subpart A of this chapter and special notification under WAC 246-290-71005 (4) and (5);

(C) If directed by the department, take corrective action as required under WAC 246-290-453(1); and

(D) Systems that are not directed by the department to take corrective action shall collect five additional samples from the same source within ((~~twenty-four~~)) 24 hours of being notified of the *E. coli* positive source water sample. If any of the five additional samples are *E. coli* positive, the system shall take corrective action under WAC 246-290-453(1).

(vi) Any consecutive groundwater system that has a total coliform-positive routine sample collected under this subsection and not invalidated under subsection (3)(d) of this section shall notify each wholesale system it receives water from within ((~~twenty-four~~)) 24 hours of being notified of the total coliform-positive sample and comply with subsection (3)(h) of this section.

(A) A wholesale groundwater system that receives notice from a consecutive system under subsection (3)(h)(vi) of this section shall conduct triggered source water monitoring under subsection (3)(h) of this section unless the department determines and documents in writing that the total coliform-positive sample collected was caused by a distribution system deficiency in the consecutive system.

(B) If the wholesale groundwater system source sample is *E. coli* positive, the wholesale system shall notify all consecutive systems served by that groundwater source within ((~~twenty-four~~)) 24 hours of being notified of the results and shall meet the requirements of subsection (3)(h)(v) of this section.

(C) Any consecutive groundwater system receiving water from a source with an *E. coli* positive sample shall notify water system users as required under subsection (3)(h)(v)(B) of this section.

(vii) An *E. coli* positive groundwater source sample may be invalidated only if one of the following conditions apply:

(A) The system provides the department with written notice from the laboratory that improper sample analysis occurred; or

(B) The department determines and documents in writing that there is substantial evidence that the *E. coli* positive groundwater sample is not related to source water quality.

(viii) If the department invalidates an *E. coli* positive groundwater source sample, the system shall collect another source water sample within ((~~twenty-four~~)) 24 hours of being notified by the department of its invalidation decision and have the sample analyzed using the same analytical method. The department may extend the ((~~twenty-four~~)) 24 hour time limit as allowed under subsection (3)(h)(iv)(A) of this section.

(ix) Groundwater systems that fail to meet any of the monitoring requirements of subsection (3)(h) of this section shall conduct Tier 2 public notification under Part 7, Subpart A of this chapter.

(i) Assessment source water monitoring. If directed by the department, a groundwater system shall conduct assessment source water monitoring which may include, but is not limited to, the collection of at least one representative groundwater source sample each month the source provides groundwater to the public, for a minimum of ((~~twelve~~)) 12 months.

(i) Sampling must be conducted as follows:

(A) Source samples must be collected at a location prior to any treatment. If the water system's configuration does not allow sampling at the source itself, the department may approve an alternative source sampling location representative of the source water quality.

(B) Source samples must be at least 100 mL in size and must be analyzed for *E. coli* using one of the analytical methods under 40 C.F.R. 141.402(c).

(ii) A groundwater system may use a triggered source water sample collected under subsection (3)(h) of this section to meet the requirements for assessment source water monitoring.

(iii) A groundwater system with an *E. coli* positive assessment source water sample that is not invalidated under subsection (3)(h)(vii) of this section, and consecutive systems receiving water from this source shall:

(A) Provide Tier 1 public notice under Part 7, Subpart A of this chapter and special notification under WAC 246-290-71005 (4) and (5); and

(B) Take corrective action as required under WAC 246-290-453(1).

(iv) A groundwater system that fails to conduct assessment source water monitoring as directed by the department shall provide Tier 2 public notice under Part 7, Subpart A of this chapter.

(4) Inorganic chemical and physical.

(a) A complete inorganic chemical and physical analysis must consist of the primary and secondary chemical and physical contaminants.

(i) Primary chemical and physical contaminants are antimony, arsenic, asbestos, barium, beryllium, cadmium, chromium, cyanide, fluoride, mercury, nickel, nitrate (as N), nitrite (as N), selenium, sodium, thallium, and for unfiltered surface water, turbidity. (Except that the MCL for arsenic under WAC 246-290-310 does not apply to TNC systems.)

(ii) Secondary chemical and physical contaminants are chloride, color, hardness, iron, manganese, specific conductivity, silver, sulfate, total dissolved solids, and zinc. Total dissolved solids are required only when specific conductivity exceeds ((~~seven hundred~~)) 700 micromhos/centimeter.

(b) Purveyors shall monitor for all primary and secondary chemical and physical contaminants identified in WAC 246-290-310, Table 5 and Table 6. Samples must be collected in accordance with the monitoring requirements referenced in 40 C.F.R. 141.23 introductory text, 141.23(a) through 141.23(j), and 40 C.F.R. 143.4, except for composite samples for systems serving less than ((~~three thousand three hundred one~~)) 3,301 persons. For these systems, compositing among different systems may be allowed if the systems are owned or operated by a department-approved satellite management agency.

(c) Samples required by this subsection must be taken at designated locations under 40 C.F.R. 141.23(a) through 141.23(j), and 40 C.F.R. 143.4, and Table 4 of this section.

(i) Wellfield samples are allowed from department designated wellfields; and

(ii) Under 40 C.F.R. 141.23 (a)(3), alternate sampling locations may be used if approved by the department. The process for determining these alternate sites is described in department guidance. Purveyors of community and NTNC systems may ask the department to approve an alternate sampling location for multiple sources within a single system that are blended prior to entry to the distribution system. Alternate sampling plans must address the following:

(A) Source vulnerability;

(B) Individual source characteristics;

(C) Previous water quality information;

(D) Status of monitoring waiver applications; and

(E) Other information deemed necessary by the department.

(d) Composite samples:

(i) Under 40 C.F.R. 141.23 (a)(4), purveyors may ask the certified laboratory to composite samples representing as many as five individual samples from within one system. Sampling procedures and protocols are outlined in department guidance; and

(ii) For systems serving a population of less than ((~~three thousand three hundred one~~)) 3,301, the department may approve composite sampling between systems when those systems are part of an approved satellite management agency.

(e) When the purveyor provides treatment for one or more inorganic chemical or physical contaminants, the department may require the purveyor to sample before and after treatment. The department shall notify the purveyor if and when this additional source sampling is required.

(f) Inorganic monitoring plans.

(i) Purveyors of community and NTNC systems shall prepare an inorganic chemical monitoring plan and base routine monitoring on the plan.

(ii) The purveyor shall:

(A) Keep the monitoring plan on file with the system and make it available to the department for inspection upon request;

(B) Revise or expand the plan at any time the plan no longer reflects the monitoring requirements, procedures or sampling locations, or as directed by the department; and

(C) Submit the plan to the department for review and approval when requested and as part of the water system plan required under WAC 246-290-100.

(g) Monitoring waivers.

(i) Purveyors may request in writing, a monitoring waiver from the department for any nonnitrate/nitrite inorganic chemical and physical monitoring requirements identified in this chapter.

(ii) Purveyors requesting a monitoring waiver shall comply with applicable subsections of 40 C.F.R. 141.23 (b)(3), and 141.23 (c)(3).

(iii) Purveyors shall update and resubmit requests for waiver renewals as applicable during each compliance cycle or period or more frequently as directed by the department.

(iv) Failure to provide complete and accurate information in the waiver application may be grounds for denial of the monitoring waiver.

(h) The department may require the purveyor to repeat sample for confirmation of results.

(i) Purveyors with emergency and seasonal sources shall monitor those sources when they are in use.

(5) Lead and copper. Monitoring for lead and copper shall be conducted in accordance with 40 C.F.R. 141.86 (a) - (f), 141.87, and 141.88. All systems that have fewer than five drinking water taps used for human consumption shall collect at least one sample from each tap and then collect additional samples from those taps on different days during the monitoring period to meet the required number of samples as described in 40 C.F.R. 141.86(c).

(6) Disinfection byproducts (DBP), disinfectant residuals, and disinfection byproduct precursors (DBPP). Purveyors of community and NTNC systems providing water treated with chemical disinfectants and TNC systems using chlorine dioxide shall monitor as follows:

(a) General requirements.

(i) Systems shall collect samples during normal operating conditions.

(ii) All monitoring shall be conducted in accordance with the analytical requirements in 40 C.F.R. 141.131.

(iii) Systems required to monitor under this subsection shall prepare and implement a monitoring plan in accordance with 40 C.F.R. 141.132(f) or 40 C.F.R. 141.622, as applicable.

(A) Community and NTNC surface water and GWI systems that deliver water that has been treated with a disinfectant other than ultraviolet light and serve more than ((~~three thousand three hundred~~)) 3,300 people shall submit a monitoring plan to the department.

(B) The department may require submittal of a monitoring plan from systems not specified in subsection (6)(a)(iii)(A) of this section, and may require revision of any monitoring plan.

(C) Failure to monitor for TTHM, HAA5, or bromate will be treated as a violation for the entire period covered by the annual average where compliance is based on a locational running annual average or

running annual average of monthly or quarterly samples or averages, as applicable.

(D) Failure to monitor for chlorine and chloramine residuals will be treated as a violation for the entire period covered by the annual average where compliance is based on a running annual average of monthly or quarterly samples or averages and the systems' failure to monitor makes it impossible to determine compliance with the MRDLs.

(b) Disinfection byproducts - Community and NTNC systems only.

(i) TTHMs and HAA5.

(A) Systems shall monitor for TTHM and HAA5 in accordance with 40 C.F.R. 141.620, 141.621, and 141.622.

(B) With department approval, systems may reduce monitoring in accordance with 40 C.F.R. 141.623.

(C) Systems on department-approved reduced monitoring schedules may be required to return to routine monitoring, or initiate increased monitoring in accordance with 40 C.F.R. 141.625 or 40 C.F.R. 141.627, as applicable.

(D) Systems must meet requirements of 40 C.F.R. 141.628 and 40 C.F.R. 141.625(c) to return to routine monitoring.

(E) Systems must calculate operational evaluation levels each calendar quarter and take action, as needed, in accordance with 40 C.F.R. 141.626.

(F) NTNC systems serving ((~~ten thousand~~)) 10,000 or more people and community systems must comply with the provisions of 40 C.F.R. Subpart U - Initial Distribution System Evaluation under:

40 C.F.R. 141.600	General requirements.
40 C.F.R. 141.601	Standard monitoring.
40 C.F.R. 141.602	System specific studies.
40 C.F.R. 141.603	40/30 certification.
40 C.F.R. 141.604	Very small system waivers.
40 C.F.R. 141.605	Subpart V compliance monitoring location recommendations.

(ii) Chlorite - Only systems that use chlorine dioxide.

(A) Systems using chlorine dioxide shall conduct daily and monthly monitoring in accordance with 40 C.F.R. 141.132 (b)(2)(i) and additional chlorite monitoring in accordance with 40 C.F.R. 141.132 (b)(2)(ii).

(B) With department approval, monthly monitoring may be reduced in accordance with 40 C.F.R. 141.132 (b)(2)(iii)(B). Daily monitoring at entry to distribution required by 40 C.F.R. 141.132 (b)(2)(i)(A) may not be reduced.

(iii) Bromate - Only systems that use ozone.

(A) Systems using ozone for disinfection or oxidation must conduct bromate monitoring in accordance with 40 C.F.R. 141.132 (b)(3)(i).

(B) With department approval, monthly bromate monitoring may be reduced to once per quarter in accordance with 40 C.F.R. 141.132 (b)(3)(ii)(B).

(c) Disinfectant residuals.

(i) Chlorine and chloramines. Systems that deliver water continuously treated with chlorine or chloramines, including consecutive systems, shall monitor and record the residual disinfectant level in the distribution system under WAC 246-290-300 (2)(b), 246-290-451, 246-290-664(6), or 246-290-694(8).

(ii) Chlorine dioxide. Community, NTNC, or TNC systems that use chlorine dioxide shall monitor in accordance with 40 C.F.R. 141.132 (c)(2) and record results.

(d) Disinfection byproducts precursors.

Community and NTNC surface water or GWI systems that use conventional filtration with sedimentation as defined in WAC 246-290-660(3) shall monitor under 40 C.F.R. 141.132(d), and meet the requirements of 40 C.F.R. 141.135.

(7) Organic chemicals.

(a) Purveyors of community and NTNC water systems shall comply with monitoring requirements under 40 C.F.R. 141.24 (f)(1) - (f)(15), 141.24 (f)(18) - (19), 141.24 (f)(21), 141.24 (h)(1) - (11), ((~~and~~)) 141.24 (h)(14) - (17)<u>, and 141.902</u>.

(b) Sampling locations shall be as defined in 40 C.F.R. 141.24(f) ((~~and~~))<u>,</u> 141.24(h)<u>, and 141.902 (a)(1) - (2)</u>.

(i) Wellfield samples are allowed from department designated wellfields; and

(ii) Under 40 C.F.R. 141.24 (f)(3) ((~~and~~))<u>,</u> 141.24 (h)(3), <u>and 141.902 (a)(1) - (2),</u> alternate sampling locations may be allowed if approved by the department. These alternate locations are described in department guidance. Purveyors may ask the department to approve an alternate sampling location for multiple sources within a single system that are blended prior to entry to the distribution system. The alternate sampling location shall consider the following:

(A) Source vulnerability;

(B) An updated organic monitoring plan showing location of all sources with current and proposed sampling locations;

(C) Individual source characteristics;

(D) Previous water quality information;

(E) Status of monitoring waiver applications; and

(F) Other information deemed necessary by the department.

(c) Composite samples:

(i) <u>With the exception of PFAS, p</u>urveyors may ask the certified lab to composite samples representing as many as five individual samples from within one system. Sampling procedures and protocols are outlined in department guidance;

(ii) For systems serving a population of less than ((~~three thousand three hundred one~~)) <u>3,301</u>, the department may approve composite sampling between systems when those systems are part of an approved satellite management agency.

(d) The department may require the purveyor to sample both before and after treatment for one or more organic contaminants. The department shall notify the purveyor if and when this additional source sampling is required.

(e) Organic chemical monitoring plans.

(i) Purveyors of community and NTNC systems shall prepare an organic chemical monitoring plan and base routine monitoring on the plan.

(ii) The purveyor shall:

(A) Keep the monitoring plan on file with the system and make it available to the department for inspection upon request;

(B) Revise or expand the plan at any time the plan no longer reflects the monitoring requirements, procedures or sampling locations, or as directed by the department; and

(C) Submit the plan to the department for review and approval when requested and as part of the water system plan required under WAC 246-290-100.

(f) Monitoring waivers.

(i) Purveyors may request in writing, a monitoring waiver from the department for any organic monitoring requirement, except for PFAS for community and NTNC systems;

(ii) Purveyors requesting a monitoring waiver shall comply with 40 C.F.R. 141.24 (f)(7), 141.24 (f)(10), 141.24 (h)(6), and 141.24 (h)(7);

(iii) Purveyors shall update and resubmit requests for waiver renewals as directed by the department; and

(iv) Failure to provide complete and accurate information in the waiver application shall be grounds for denial of the monitoring waiver.

(g) Purveyors with emergency and seasonal sources shall monitor those sources under the applicable requirements of this section when they are actively providing water to consumers.

(8) Radionuclides. Monitoring for radionuclides shall be conducted under 40 C.F.R. 141.26.

(9) *Cryptosporidium* and *E. coli* source monitoring. Purveyors with surface water or GWI sources shall monitor the sources in accordance with 40 C.F.R. 141.701 and 702.

(10) Contaminants with a SAL under WAC 246-290-315, Table 9.

(a) Purveyors shall monitor for contaminants with a SAL in accordance with Tables 3 and 4 of this section. Source sample locations and blended samples are allowed as consistent with other federally regulated organic contaminants referenced in subsection (7)(b) of this section.

TABLE 3
SAL MONITORING

Contaminant or Group of Contaminants	Applicable Water System Classification	Initial Sampling	Routine Sampling Frequency	Sampling Location
Organic Contaminants				
Per- and polyfluoroalkyl substances (PFAS)	Community and NTNC, and if applicable, TNC	One sample on or before December 31, 2025	Once every three years	Per the locations described in WAC 246-290-300 (7)(b) and Table 4 of this section

(b) Purveyors shall monitor for PFAS contaminants using an approved method in WAC 246-390-075 (17)(a) and all method specific contaminants as listed on Table 7 in WAC 246-390-075.

(i) Purveyors, on a schedule determined by the department, but no later than December 31, 2025, shall complete initial sampling for PFAS from a sample location representing each source as listed in Table 4 of this section.

(ii) Initial PFAS sampling prioritization and scheduling is based on the following criteria:

(A) Susceptibility of the source water to contamination by surface activities due to physical attributes of the source;

(B) Vulnerability of the source water to PFAS contamination; and

(C) Population served.

(iii) Purveyors of TNC systems determined by the department to be at risk of PFAS contamination due to proximity of the system's water supply to known PFAS contamination shall collect a sample for analysis as directed by the department and, if detected, comply with the follow-up requirements under WAC 246-290-320(8).

(c) Analytical results for contaminants or groups of contaminants listed in Table 3 of this section reported prior to January 1, 2023, that meet the SDRL and requirements established in chapter 246-390 WAC with the effective date of January 1, 2022, or later may be used to comply with the initial PFAS monitoring requirement in this chapter.

(d) For sources that become active after January 1, 2022, purveyors shall perform the required monitoring as part of the source approval process under WAC 246-290-130.

(e) The department may require a confirmation sample. If a confirmation sample is required by the department, the result will be averaged with the first sampling result and the average is used as the final result. The department has the discretion to delete results of obvious sampling errors from this calculation.

(f) After completing initial sampling as described in Table 3 of this section, each source must be monitored as follows:

(i) For sources with organic results less than the SDRL, purveyors shall begin routine monitoring as described in Table 3 of this section, unless a monitoring waiver is granted by the department under (h) of this subsection.

(ii) For sources with organic detections equal to or greater than the SDRL, purveyors shall conduct follow-up monitoring under WAC 246-290-320(8).

(iii) For sources with inorganic detections below the SAL, purveyors shall monitor as identified in subsection (4) of this section.

(iv) For sources with inorganic detections above the SAL, purveyors shall conduct follow-up monitoring under WAC 246-290-320(9).

(g) For public water systems required to sample for PFAS under EPA's fifth Unregulated Contaminant Monitoring Rule (UCMR5), if the minimum reporting limits are less than ((~~fifty~~)) 50 percent of any SAL, the department shall accept data that has been accepted by EPA under UCMR5 for the purposes of meeting initial monitoring requirements under WAC 246-290-300. Public water systems required to sample for PFAS shall submit all results to the department to be considered in compliance with the initial monitoring requirements.

(i) If the minimum reporting limit for a result is greater than ((~~twenty~~)) 20 percent of any SAL, but the result is reported as a nondetect, then follow-up monitoring per WAC 246-290-320 will not be required.

(ii) If a detection is reported below the minimum detection limit and is greater than ((~~twenty~~)) 20 percent of a SAL, then one additional quarter of follow-up monitoring will be required per WAC 246-290-320 (8)(b).

(h) Monitoring waivers for contaminants with a SAL.

(i) The department may grant a waiver for SAL monitoring requirements identified in this chapter.

(ii) As a condition of the waiver, the department may require a purveyor take a minimum of one sample per source while the waiver is effective.

(11) Other contaminants. On the basis of public health concerns, the department may require a purveyor to monitor for additional contaminants.

TABLE 4
MONITORING LOCATION

Sample Type	Sample Location
Asbestos	One sample from distribution system or if required by department, from the source.
Bacteriological	From representative points throughout distribution system.
Cryptosporidium and *E. coli* (Source Water) - WAC 246-290-630(16)	Under 40 C.F.R. 141.703.
Complete Inorganic Chemical & Physical	From a point representative of the source, after treatment, and prior to entry to the distribution system.
Lead/Copper	From the distribution system at targeted sample tap locations.
Nitrate/Nitrite	From a point representative of the source, after treatment, and prior to entry to the distribution system.
Disinfection Byproducts - TTHMs and HAA5 - WAC 246-290-300(6)	Under 40 C.F.R. 141.132 (b)(1) (Subpart L of the C.F.R.).
Disinfection Byproducts - TTHMs and HAA5 - WAC 246-290-300(6)	Under 40 C.F.R. 141.600 - 629 (IDSE and LRAA in Subparts U and V of the C.F.R.).
Disinfection Byproducts - Chlorite (Systems adding chlorine dioxide)	Under 40 C.F.R. 141.132 (b)(2).
Disinfection Byproducts - Bromate (Systems adding ozone)	Under 40 C.F.R. 141.132 (b)(3).
Disinfectant Residuals - Chlorine and Chloramines	Under 40 C.F.R. 141.132 (c)(1).
Disinfectant Residuals - Chlorine dioxide	Under 40 C.F.R. 141.132 (c)(2).
Disinfection Precursors - Total Organic Carbon (TOC)	Under 40 C.F.R. 141.132(d).
Disinfection Precursors - Bromide (Systems using ozone)	From the source before treatment.
Radionuclides	From a point representative of the source, after treatment and prior to entry to distribution system.
Organic Chemicals (VOCs, SOCs, & PFAS)	From a point representative of the source, after treatment and prior to entry to distribution system.
Other contaminants without an MCL, MRDL, TT or SAL	As directed by the department.

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-310 Maximum contaminant levels (MCLs) and maximum residual disinfectant levels (MRDLs). (1) General.

(a) The purveyor shall be responsible for complying with the standards of water quality identified in this section. If a contaminant exceeds its MCL or its maximum residual disinfectant level (MRDL), the purveyor shall take follow-up action under WAC 246-290-320.

(b) When enforcing the standards described under this section, the department shall enforce compliance with the primary standards as its first priority.

(2) Bacteriological.

(a) An *E. coli* MCL under this subsection is considered a primary standard.

(b) *E. coli* MCL. An *E. coli* MCL violation occurs each month in which a system is required to monitor for total coliforms when there is:

(i) *E. coli* presence in a repeat sample following a total coliform presence routine sample;

(ii) Total coliform presence in any repeat samples collected as a follow-up to a sample with *E. coli* presence;

(iii) The system fails to take all required repeat samples following an *E. coli* presence routine sample; or

(iv) The system fails to test for *E. coli* when any repeat samples test positive for total coliform.

(3) Inorganic chemical and physical.

(a) The primary and secondary standards are listed in Tables 5 and 6 of this section:

TABLE 5
INORGANIC CHEMICAL CHARACTERISTICS

Contaminant	Primary MCLs (mg/L)
Antimony (Sb)	0.006
Arsenic (As)	0.010*
Asbestos	7 million fibers/liter (longer than 10 microns)
Barium (Ba)	2.0
Beryllium (Be)	0.004
Cadmium (Cd)	0.005
Chromium (Cr)	0.1
Copper (Cu)	**
Cyanide (CN)	0.2
Fluoride (F)	4.0***
Lead (Pb)	**
Mercury (Hg)	0.002
Nitrate (as N)	10.0
Nitrite (as N)	1.0
Selenium (Se)	0.05
Sodium (Na)	**

Thallium (Tl)	0.002

Contaminant	Secondary MCLs (mg/L)
Chloride (Cl)	250.0
Fluoride (F)	2.0***
Iron (Fe)	0.3
Manganese (Mn)	0.05
Silver (Ag)	0.1
Sulfate (SO_4)	250.0
Zinc (Zn)	5.0

Note* Does not apply to TNC systems.

Note** Although the state board of health has not established MCLs for copper, lead, and sodium, there is sufficient public health significance connected with copper, lead, and sodium levels to require inclusion in inorganic chemical and physical source monitoring. For lead and copper, the EPA has established distribution system related levels at which a system is required to consider corrosion control. These levels, called "action levels," are 0.015 mg/L for lead and 1.3 mg/L for copper and are applied to the highest concentration in ((~~ten~~)) 10 percent of all samples collected from the distribution system. The EPA has also established a recommended level of ((~~twenty~~)) 20 mg/L for sodium as a level of concern for those consumers that may be restricted for daily sodium intake in their diets.

Note*** If a water system provides community fluoridation, the level of fluoride and associated requirements are set under WAC 246-290-460.

TABLE 6
PHYSICAL CHARACTERISTICS

Contaminant	Secondary MCLs
Color	15 Color Units
Specific Conductivity	700 umhos/cm
Total Dissolved Solids (TDS)	500 mg/L

(b) Compliance with the MCLs, except for nitrate and nitrite, in this subsection is determined by a running annual average at each sampling point. The system will not be considered in violation of the MCL until it has completed one year of quarterly sampling and at least one sampling point is in violation of the MCL. If one sampling point is in violation of the MCL, the system is in violation of the MCL.

(i) If any sample will cause the running annual average to exceed the MCL at any sampling point, the system is out of compliance with the MCL immediately.

(ii) If a system fails to collect the required number of samples, compliance will be based on the total number of samples collected.

(iii) If a sample result is less than the detection limit, zero will be used to calculate the running annual average.

(c) Compliance with the MCLs for nitrate and nitrite is determined based on one sample if the levels of these contaminants are below the MCLs as determined under Table 5 of this section. If the levels of nitrate or nitrite exceed the MCLs in the initial sample, a confirmation sample is required under 40 C.F.R. 141.23 (f)(2), and compliance shall be determined based on the average of the initial and confirmation samples.

(4) Disinfection byproducts.

(a) The department shall consider standards under this subsection as primary standards. The MCLs in this subsection apply to monitoring required by WAC 246-290-300(6) and 40 C.F.R. 141.620 - 629.

(b) The MCLs for disinfection byproducts are as follows:

TABLE 7
DISINFECTION BYPRODUCTS

Disinfection Byproduct	MCL (mg/L)
Total Trihalomethanes (TTHMs)	0.080
Haloacetic acids (five) (HAA5)	0.060
Bromate	0.010
Chlorite	1.0

(c) Whether a system has exceeded the disinfection byproduct MCLs shall be determined in accordance with 40 C.F.R. 141.133. Beginning on the dates specified for compliance in 40 C.F.R. 141.620(c), compliance with the TTHMs and HAA5 MCLs shall be based on the LRAAs as required by 40 C.F.R. 141.64 (b)(2) and 40 C.F.R. 141.620(d). Compliance with the bromate and chlorite MCL will continue to be determined in accordance with 40 C.F.R. 141.133.

(5) Disinfectant residuals.

(a) The department shall consider standards under this subsection primary standards. The MRDLs in this subsection apply to monitoring required by WAC 246-290-300(6).

(b) The MRDL for disinfectants is as follows:

TABLE 8
DISINFECTANT RESIDUAL MRDLs

Disinfectant Residual	MRDL (mg/L)
Chlorine	4.0 (as Cl_2)
Chloramines	4.0 (as Cl_2)
Chlorine Dioxide	0.8 (as ClO_2)

(c) Whether a system has exceeded MRDLs shall be determined in accordance with 40 C.F.R. 141.133.

(6) Radionuclides.

(a) The department shall consider standards under this subsection primary standards.

(b) The MCLs for radium-226 and radium-228, gross alpha particle activity, beta particle and photon radioactivity, and uranium shall be as listed in 40 C.F.R. 141.66.

(7) Organic chemicals.

(a) The department shall consider standards under this subsection primary standards.

(b) VOCs.

(i) The MCLs for VOCs are as follows:

Contaminant	Chemical Abstract Service (CAS) Number	MCL (ppb)
Vinyl chloride	75–01–4	2
Benzene	71–43–2	5
Carbon tetrachloride	56–23–5	5
1,2-Dichloroethane	107–06–2	5
Trichloroethylene	79–01–6	5
para-Dichlorobenzene	106–46–7	75
1,1-Dichloroethylene	75–35–4	7
1,1,1-Trichloroethane	71–55–6	200
cis-1,2-Dichloroethylene	156–59–2	70
1,2-Dichloropropane	78–87–5	5

Contaminant	Chemical Abstract Service (CAS) Number	MCL (ppb)
Ethylbenzene	100–41–4	700
Monochlorobenzene	108–90–7	100
o-Dichlorobenzene	95–50–1	600
Styrene	100–42–5	100
Trichloroethane	127–18–4	5
Toluene	108–88–3	1,000
trans-1,2-Dichloroethylene	156–60–5	100
Xylenes (total)	1330–20–7	10,000
Dichloromethane	75–09–2	5
1,2,4-Trichlorobenzene	120–82–1	70
1,1,2-Trichloroethane	79–00–5	5

(ii) The department shall determine compliance with this subsection based on compliance with 40 C.F.R. 141.24(f).

(c) SOCs, except PFAS.

(i) MCLs for SOCs shall be as listed in 40 C.F.R. 141.61(c).

(ii) The department shall determine compliance with this subsection based on compliance with 40 C.F.R. 141.24(h).

(d) PFAS.

(i) The MCLs for PFAS, effective in accordance with 40 C.F.R. 141.6, are as follows:

Contaminant	Chemical Abstract Service (CAS) Number	MCL (ppt or ng/L)	HBWC (ppt or ng/L) for hazard index calculation
PFOA	45285–51–6	4.0	Not applicable
PFOS	45298–90–6	4.0	Not applicable
PFHxS	108427–53–8	10	10
HFPO-DA	122499–17–6	10	10
PFNA	72007–68–2	10	10
PFBS	45187–15–3	No individual MCL	2,000
Hazard Index PFAS (HFPO-DA, PFBS, PFHxS, and PFNA)	Not applicable	1 (unitless)[1]	Not applicable

[1]The PFAS Mixture Hazard Index (HI) is the sum of component hazard quotients (HQs), which are calculated by dividing the measured component PFAS concentration in water by the relevant health-based water concentration when expressed in the same units (shown in ppt for simplification). The HBWC for PFHxS is 10 ng/l; the HBWC for HFPO-DA is 10 ng/l; the HBWC for PFNA is 10 ng/l; and the HBWC for PFBS is 2000 ng/l.

(ii) The MCLs for PFAS will become effective according to the dates outlined in 40 C.F.R. 141.6.

(8) Other contaminants.

The state board of health shall determine state MCLs for any additional contaminants as described in WAC 246-290-315 (5) through (8).

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-320 Follow-up action. (1) General.

(a) When an MCL or MRDL violation or SAL exceedance occurs, the purveyor shall take follow-up action as described in this section.

(b) When a primary standard violation occurs the purveyor shall:

(i) Notify the department under WAC 246-290-480;

(ii) Notify the consumers served by the system and the owner or operator of any consecutive system served in accordance with 40 C.F.R. 141.201 through 208, and Part 7, Subpart A of this chapter;

(iii) Investigate the cause of the contamination, within the purveyor's control; and

(iv) Take action as directed by the department.

(c) When a secondary standard violation occurs, the purveyor shall notify the department and take action as directed by the department.

(d) When a SAL exceedance under WAC 246-290-315 occurs, the purveyor shall:

(i) Notify the department in accordance with WAC 246-290-480;

(ii) Notify water system users and the owner or operator of any consecutive system served water in exceedance of the SAL in accordance with WAC 246-290-71006;

(iii) Continue monitoring in accordance with subsection (8) or (9) of this section, as applicable;

(iv) Investigate the cause of the contamination within the purveyor's control; and

(v) Take action as directed by the department.

(e) The department may require additional sampling for confirmation of results.

(2) Bacteriological. Coliform treatment technique triggers and assessment requirements for protection against potential fecal contamination.

(a) Treatment technique triggers. Systems shall conduct assessments in accordance with (b) of this subsection after exceeding treatment technique triggers as follows:

(i) Level 1 treatment technique triggers.

(A) For systems taking ((~~forty~~)) 40 or more routine samples per month, the system exceeds 5.0 percent total coliform-positive samples for the month.

(B) For systems taking fewer than ((~~forty~~)) 40 routine samples per month, the system has two or more total coliform-positive samples in the same month.

(C) The system fails to take every required repeat sample after any single total coliform-positive routine sample.

(ii) Level 2 treatment technique triggers.

(A) An *E. coli* MCL violation, as specified in WAC 246-290-310 (2)(b).

(B) A second level 1 treatment technique trigger as defined in (a)(i) of this subsection within a rolling ((~~twelve~~)) 12-month period, unless the department has determined a likely reason that the samples that caused the first level 1 treatment technique trigger were total coliform-positive and has established that the system has corrected the problem.

(b) Requirements for assessments.

(i) Systems shall conduct level 1 and 2 assessments to identify the possible presence of sanitary defects and defects in distribution system coliform monitoring practices. Level 1 assessments must be conducted by the system operator or purveyor. Level 2 assessments must be conducted by the department or a party approved by the department which may include the system operator.

(ii) When conducting assessments, systems shall direct the assessor to evaluate minimum elements that include:

(A) Review and identification of atypical events that could affect distributed water quality or indicate that distributed water quality was impaired;

(B) Changes in distribution operation and maintenance that could affect distributed water quality, including water storage;

(C) Source and treatment considerations that bear on distributed water quality, where appropriate. For example, whether or not a groundwater system is disinfected;

(D) Existing water quality monitoring data;

(E) Inadequacies in sample sites, sampling protocol, and sample processing; and

(F) The system shall conduct the assessment consistent with any department directives that tailor specific assessment elements with respect to the size and type of the system and the size, type, and characteristics of the distribution system.

(iii) Level 1 assessments. A system shall conduct a level 1 assessment consistent with the requirements in subsection (2)(b) of this section if the system exceeds one of the treatment technique triggers in (a)(i) of this subsection.

(A) The system shall complete a level 1 assessment as soon as practical after any treatment technique trigger is met in (a)(i) of this subsection. The completed assessment must describe sanitary defects detected, corrective actions completed, and a proposed timetable for any corrective actions not already completed. The assessment may also note that no sanitary defects were identified. The system shall submit the completed level 1 assessment to the department within ((~~thirty~~)) 30 days after the system learns that it has exceeded a treatment technique trigger.

(B) Upon completion and submission of the level 1 assessment by the system, the department shall determine if the system has identified a likely cause for the level 1 treatment technique trigger and has corrected the problem. If the system has not corrected the problem, the department shall determine if the proposed timetable for corrective action is sufficient.

(C) If after reviewing the completed level 1 assessment, the department determines the assessment is not sufficient, including any proposed timetable for any corrective actions not already completed, the department may require the system to submit a revised assessment to the department within ((~~thirty~~)) 30 days from the date of department notification.

(iv) Level 2 assessments. A system shall conduct a level 2 assessment consistent with requirements in subsection (2)(b) of this section if the system exceeds one of the treatment technique triggers in (a)(ii) of this subsection. The system shall comply with any expedited actions or additional actions required by the department in the case of an *E. coli* MCL violation.

(A) A level 2 assessment must be conducted as soon as practical after any treatment technique trigger in (a)(ii) of this subsection and shall be conducted by either a water distribution manager 2, 3, or 4 certified in accordance with chapter 246-292 WAC, a licensed professional engineer that meets the requirements of WAC 246-290-040(1), a local health jurisdiction, or the department. The system shall submit a completed level 2 assessment to the department within ((~~thirty~~)) 30 days after the system learns that it has exceeded a treatment technique trigger. The completed assessment must describe sanitary defects detected, corrective actions completed, and a proposed timetable for any corrective actions not already completed in accordance with (d) of

this subsection. The assessment may also note that no sanitary defects were identified.

(B) Upon completion and submission of the level 2 assessment by the system, the department shall determine if the system has identified a likely cause for the level 2 treatment technique trigger and has corrected the problem. If the system has not corrected the problem, the department shall determine if the proposed timetable for corrective action is sufficient.

(C) If after reviewing the submitted level 2 assessment, the department determines the assessment is not sufficient, including any proposed timetable for any corrective actions not already completed in accordance with (d) of this subsection, the department may require the system to submit a revised assessment within ((~~thirty~~)) 30 days from the date of department notification.

(c) To achieve compliance with the MCL for *E. coli* under WAC 246-290-310 (2)(b), the following are identified as the best technology, treatment techniques, or other means available:

(i) Protection of wells from fecal contamination by appropriate placement and construction;

(ii) Maintenance of a disinfectant residual throughout the distribution system;

(iii) Proper maintenance of the distribution system including appropriate pipe replacement and repair procedures, main flushing programs, proper operation and maintenance of storage tanks and reservoirs, cross-connection control, and continual maintenance of positive water pressure in all parts of the distribution system;

(iv) Filtration, disinfection, or both, of surface water, using the proper strength of oxidants such as chlorine, chlorine dioxide, or ozone; and

(v) For systems using groundwater, compliance with a wellhead protection program developed and implemented under WAC 246-290-135(3).

(d) Corrective action. Systems shall correct sanitary defects found through either a level 1 or level 2 assessment conducted under (b) of this subsection. For corrections not completed by the time of submission of the assessment to the department, the system shall complete the corrective actions in compliance with a timetable approved by the department in consultation with the system under (e) of this subsection. The system shall notify the department when each scheduled corrective action is completed.

(e) Consultation. At any time during the assessment or corrective action phase, the water system may request a consultation with the department to determine the appropriate actions to be taken. The system may consult with the department on all relevant information that may impact the system's ability to comply with the requirements of subsection (2) of this section, including the method of accomplishment, an appropriate time frame, and other relevant information.

(f) A treatment technique violation occurs when a system exceeds a treatment technique trigger specified in subsection (2)(a) of this section and then fails to conduct the required assessment or complete corrective actions within the time frame specified in subsection (2)(b) and (d) of this section.

(3) Inorganic chemical and physical follow-up monitoring shall be conducted in accordance with the following:

(a) For nonnitrate/nitrite primary inorganic chemicals, 40 C.F.R. 141.23 (a)(4), 141.23 (b)(8), 141.23 (c)(7), 141.23 (c)(9), 141.23 (f)(1), 141.23(g), 141.23(m) and 141.23(n);

(b) For nitrate, 40 C.F.R. 141.23 (a)(4), 141.23 (d)(2), 141.23 (d)(3), 141.23 (f)(2), 141.23(g), 141.23(m), 141.23(n), and 141.23(o);

(c) For nitrite, 40 C.F.R. 141.23 (a)(4), 141.23 (e)(3), 141.23 (f)(2), and 141.23(g); or

(d) The purveyor of any public water system providing service that has secondary inorganic MCL exceedances shall take follow-up action as required by the department. Follow-up action shall be commensurate with the degree of consumer acceptance of the water quality and their willingness to bear the costs of meeting the secondary standard. For new community water systems and new nontransient noncommunity water systems without active consumers, treatment for secondary contaminant MCL exceedances will be required.

(4) Lead and copper follow-up monitoring shall be conducted in accordance with 40 C.F.R. 141.85(c), 141.86 (d)(2), 141.86 (d)(3), 141.87(c), 141.87(d) and 141.88(b) through 141.88(d).

(5) Turbidity.

Purveyors monitoring turbidity in accordance with Part 6 of this chapter shall provide follow-up under WAC 246-290-634.

(6) Organic chemicals. Follow-up monitoring shall be conducted in accordance with the following:

(a) For VOCs, 40 C.F.R. 141.24 (f)(11) through 141.24 (f)(15), and 141.24 (f)(22); or

(b) For SOCs, 40 C.F.R. 141.24 (h)(7) through 141.24 (h)(11), ((~~and~~)) 141.24 (h)(20)<u>, 141.902 (b)(2), and 141.903</u>.

(7) Radionuclide follow-up monitoring shall be conducted under 40 C.F.R. 141.26 (a)(2)(iv), 141.26 (a)(3)(ii) through (v), 141.26 (a)(4), 141.26 (b)(6), and 141.26 (c)(5).

(8) Organic contaminants with a SAL.

(a) All increased monitoring for organic contaminants will be for the test panel on which the contaminant is listed in WAC 246-390-075.

(b) The purveyor shall monitor quarterly as shown in Table 10 of this section. The number of samples required in the three quarters after the first detection will be determined based on the highest detection in the year of monitoring. For public water systems required to sample for PFAS under the federal Unregulated Contaminant Monitoring Rule 5 (UCMR5), if a detection is reported below the minimum detection limit per UCMR5 and is greater than ((~~twenty~~)) <u>20</u> percent of a SAL, then one additional quarter of follow-up monitoring is required. The samples must be taken:

(i) Beginning in the calendar quarter following the first confirmed detection per WAC 246-290-315 (4)(b); and

(ii) At each sampling point from which the result was equal to or greater than the SDRL under chapter 246-390 WAC.

TABLE 10
MONITORING REQUIREMENTS FOLLOWING THE FIRST DETECTION OF AN ORGANIC CONTAMINANT WITH A SAL

If the highest detection in the first year is:	Total number of additional consecutive quarters.
≤ 20% of the SAL.	1
> 20% but < 80% of the SAL.	2
≥ 80% of the SAL.	3

(c) Ongoing monitoring is specified in Table 11 of this section, or as directed by the department. Ongoing monitoring is based upon the results of samples collected in the most recent year of monitoring, or

the most recent result for samples collected less frequently than annually.

TABLE 11
ONGOING MONITORING REQUIREMENTS FOR SOURCES WITH ORGANIC CONTAMINANTS WITH A SAL

If highest detection being considered is:	Monitoring frequency:
≤ 20% of the SAL.	Every 3 years
> 20% but < 80% of the SAL.	Annually
≥ 80% of the SAL.	1. Quarterly, if contaminant is Tier 1, or Tier 2 and bioaccumulative per Table 17 in WAC 246-290-71006. 2. Annually if the contaminant is Tier 2 and not bioaccumulative per Table 17 in WAC 246-290-71006.

(d) When the monitoring frequency is less often than quarterly, the purveyor shall collect samples during the quarter assigned by the department.

(e) The department may reduce the annual monitoring frequency to one sample every three years after three consecutive years of results that demonstrate the levels are less than ((~~eighty~~)) 80 percent of the SAL.

(f) The department may increase the monitoring frequency from once every year or once every three years to once every quarter if results of reduced monitoring are equal to or greater than ((~~eighty~~)) 80 percent of the SAL.

(9) Inorganic contaminants with a SAL.

(a) The purveyor shall collect quarterly samples at each sampling point beginning in the quarter following a detection greater than the SAL under Table 9 of this section. Increased monitoring for inorganic contaminants will be for a specific contaminant which is detected above the SAL.

(b) The department may reduce the quarterly monitoring frequency when results are reliably and consistently below the SAL. When the monitoring frequency is less often than quarterly, the purveyor shall collect samples during the quarter assigned by the department.

(10) If a contaminant has no MCL, MRDL, TT, or SAL, the department may use an EPA *health advisory level* to determine subsequent monitoring per this section.

(11) The department shall determine the purveyor's follow-up action when a contaminant not included in this chapter is detected.

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-71002 Public notice content. (1) Public notices required under WAC 246-290-71001(1) must contain the elements and standard language required under 40 C.F.R. 141.205 (a), (b), and (d) and be

presented in accordance with 40 C.F.R. 141.205 (c), except that notification of the availability of results required per 40 C.F.R. 141.40 and notification of an exceedance of the secondary MCL for fluoride must be in accordance with WAC 246-290-71005.

(2) Public notices required under WAC 246-290-71001 (3)(a) and (c) for the issuance of a departmental order or category red operating permit must include:

(a) A clear, concise, and simple explanation of the violation;

(b) Discussion of potential adverse health effects and any segments of the population that may be at higher risk;

(c) Mandatory health effects information in accordance with WAC 246-290-71004(2);

(d) A list of steps the purveyor has taken or is planning to take to remedy the situation;

(e) A list of steps the consumer should take, including advice on seeking an alternative water supply if necessary;

(f) The purveyor's name and telephone number; and

(g) When appropriate, notices must be bilingual or multilingual.

(3) The purveyor may provide additional information to further explain the situation.

((~~Note: The purveyor may provide additional information to further explain the situation.~~))

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-72004 Report contents—Definitions. (1) Each report must include the following definitions:

(a) Maximum contaminant level goal or MCLG: The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs allow for a margin of safety.

(b) Maximum contaminant level or MCL: The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to the MCLGs as feasible using the best available treatment technology.

(2) A report for a community water system operating under a variance or an exemption issued under WAC 246-290-060 must include the following definition: Variances and exemptions: State or EPA permission not to meet an MCL or a treatment technique under certain conditions.

(3) A report that contains data on contaminants that the Environmental Protection Agency regulates using any of the following terms must include the applicable definitions:

(a) Treatment technique: A required process intended to reduce the level of a contaminant in drinking water.

(b) Action level: The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow.

(c) Maximum residual disinfectant level goal or MRDLG: The level of a drinking water disinfectant below which there is no known or expected risk to health. MRDLGs do not reflect the benefits of the use of disinfectants to control microbial contaminants.

(d) Maximum residual disinfectant level or MRDL: The highest level of a disinfectant allowed in drinking water. There is convincing

evidence that addition of a disinfectant is necessary for control of microbial contaminants.

(e) Hazard index or HI: The hazard index is an approach that determines the health concerns associated with mixtures of certain PFAS in finished drinking water. Low levels of multiple PFAS that individually would not likely result in adverse health effects may pose health concerns when combined in a mixture. The hazard index MCL represents the maximum level for mixtures of PFHxS, PFNA, HFPO-DA, and/or PFBS allowed in water delivered by a public water system. A hazard index greater than 1 requires a system to take action.

(4) A report that contains level 1 or level 2 assessment information must include the applicable definitions:

(a) Level 1 assessment: A level 1 assessment is a study of the water system to identify potential problems and determine, if possible, why total coliform bacteria have been found in our water system.

(b) Level 2 assessment: A level 2 assessment is a very detailed study of the water system to identify potential problems and determine, if possible, why an *E. coli* MCL violation has occurred and, if applicable, why total coliform bacteria have been found in our water system on multiple occasions.

(5) A report that contains information regarding a detection of a contaminant with a SAL must include the following definition: State action level (SAL) means the concentration of a contaminant or group of contaminants, without an MCL, in drinking water established to protect public health and which, if exceeded, triggers actions a water system purveyor must take. SALs are established for contaminants without an MCL, federal action level, or treatment technique.

AMENDATORY SECTION (Amending WSR 21-23-097, filed 11/17/21, effective 1/1/22)

WAC 246-290-72012 Regulated contaminants.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Microbiological Contaminants						
Total Coliform Bacteria	TT	-	TT	0	Naturally present in the environment	Coliforms are bacteria that are naturally present in the environment and are used as an indicator that other, potentially harmful, waterborne pathogens may be present or that a potential pathway exists through which contamination may enter the drinking water distribution system. We found coliforms indicating the need to look for potential problems in water treatment or distribution. When this occurs, we are required to conduct assessment(s) to identify problems and to correct any problems that were found during these assessments.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
E. coli	Routine and repeat samples are total coliform-positive and either is *E. coli*-positive or system fails to take repeat samples following *E. coli*-positive routine sample or system fails to analyze total coliform-positive repeat sample for *E. coli*.	-	Routine and repeat samples are total coliform-positive and either is *E. coli*-positive or system fails to take repeat samples following *E. coli*-positive routine sample or system fails to analyze total coliform-positive repeat sample for *E. coli*.	0	Human and animal fecal waste	*E. coli* are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Human pathogens in these wastes can cause short-term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a greater health risk for infants, young children, the elderly, and people with severely-compromised immune systems.
Fecal indicators (*E. coli*)	TT	-	TT	0	Human and animal fecal waste	Fecal indicators are microbes whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term health effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, some of the elderly, and people with severely compromised immune systems.
Total organic carbon	TT	-	TT	N/A	Naturally present in the environment	Total organic carbon (TOC) has no health effects. However, total organic carbon provides a medium for the formation of disinfection byproducts. These byproducts include trihalomethanes (THMs) and haloacetic acids (HAAs). Drinking water containing these byproducts in excess of the MCL may lead to adverse health effects, liver or kidney problems, or nervous system effects, and may lead to an increased risk of getting cancer.
Turbidity	TT	-	TT	N/A	Soil runoff	Turbidity has no health effects. However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease-causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhea and associated headaches.
Giardia lamblia Viruses *Cryptosporidium*	TT	-	TT	0	Human and animal fecal waste	Inadequately treated water may contain disease-causing organisms. These organisms include bacteria viruses, and parasites which can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.
Heterotrophic plate count (HPC) bacteria	TT	-	TT	N/A	HPC measures a range of bacteria that are naturally present in the environment	Inadequately treated water may contain disease-causing organisms. These organisms include bacteria viruses, and parasites which can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Legionella	TT	-	TT	0	Found naturally in water; multiplies in heating systems	Inadequately treated water may contain disease-causing organisms. These organisms include bacteria viruses, and parasites which can cause symptoms such as nausea, cramps, diarrhea, and associated headaches.
Radioactive Contaminants						
Beta/photon emitters	4 mrem/yr	-	4 mrem/yr	0	Decay of natural and man-made deposits	Certain minerals are radioactive and may emit forms of radiation known as photons and beta radiation. Some people who drink water containing beta and photon emitters in excess of the MCL over many years may have an increased risk of getting cancer.
Alpha emitters [gross alpha excluding uranium and radon]	15 pCi/l	-	15 (pCi/L)	0	Erosion of natural deposits	Certain minerals are radioactive and may emit a form of radiation known as alpha radiation. Some people who drink water containing alpha emitters in excess of the MCL over many years may have an increased risk of getting cancer.
Combined radium [226 & 228]	5 pCi/l	-	5 (pCi/L)	0	Erosion of natural deposits	Some people who drink water containing radium 226 or 228 in excess of the MCL over many years may have an increased risk of getting cancer.
Uranium	30 ppb	-	30 ppb	0	Erosion of natural deposits	Some people who drink water containing uranium in excess of the MCL over many years may have an increased risk of getting cancer and kidney toxicity.
Inorganic Contaminants						
Antimony	.006 ppm	1000	6 ppb	6 ppb	Discharge from petroleum refineries; fire retardants; ceramics; electronics; solder	Some people who drink water containing antimony well in excess of the MCL over many years could experience increases in blood cholesterol and decreases in blood sugar.
Arsenic	0.010 ppm	1000	10 ppb	0	Erosion of natural deposits; Runoff from orchards; Runoff from glass and electronics production wastes	Some people who drink water containing arsenic in excess of the MCL over many years could experience skin damage or problems with their circulatory system, and may have an increased risk of getting cancer.
Asbestos	7 MFL	-	7 MFL	7 MFL	Decay of asbestos cement water mains; Erosion of natural deposits	Some people who drink water containing asbestos in excess of the MCL over many years may have an increased risk of developing benign intestinal polyps.
Barium	2 ppm	-	2 ppm	2 ppm	Discharge of drilling wastes; Discharge from metal refineries; Erosion of natural deposits	Some people who drink water containing barium in excess of the MCL over many years could experience an increase in their blood pressure.
Beryllium	.004 ppm	1000	4 ppb	4 ppb	Discharge from metal refineries and coal-burning factories; Discharge from electrical, aerospace, and defense industries	Some people who drink water containing beryllium well in excess of the MCL over many years could develop intestinal lesions.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Cadmium	.005 ppm	1000	5 ppb	5 ppb	Corrosion of galvanized pipes; Erosion of natural deposits; Discharge from metal refineries; Runoff from waste batteries and paints	Some people who drink water containing cadmium in excess of the MCL over many years could experience kidney damage.
Chromium	.1 ppm	1000	100 ppb	100 ppb	Discharge from steel and pulp mills; Erosion of natural deposits	Some people who use water containing chromium well in excess of the MCL over many years could experience allergic dermatitis.
Copper	TT AL = 1.3 ppm	-	TT AL = 1.3 ppm	1.3 ppm	Corrosion of household plumbing systems; Erosion of natural deposits	Copper is an essential nutrient, but some people who drink water containing copper in excess of the action level over a relatively short amount of time could experience gastrointestinal distress. Some people who drink water containing copper in excess of the action level over many years could suffer liver or kidney damage. People with Wilson's Disease should consult their personal doctor.
Cyanide	.2 ppm	1000	200 ppb	200 ppb	Discharge from steel/metal factories; Discharge from plastic and fertilizer factories	Some people who drink water containing cyanide well in excess of the MCL over many years could experience nerve damage or problems with their thyroid.
Fluoride	4 ppm	-	4 ppm	4 ppm	Erosion of natural deposits; Water additive which promotes strong teeth; Discharge from fertilizer and aluminum factories	Some people who drink water containing fluoride in excess of the MCL over many years could get bone disease, including pain and tenderness of the bones. Fluoride in drinking water at half the MCL or more may cause mottling of children's teeth, usually in children less than nine years old. Mottling, also known as dental fluorosis, may include brown staining and/or pitting of the teeth, and occurs only in developing teeth before they erupt from the gums.
Lead	TT AL = .015 ppm	1000	TT AL = 15 ppb	0	Corrosion of household plumbing systems; Erosion of natural deposits	Infants and children who drink water containing lead in excess of the action level could experience delays in their physical or mental development. Children could show slight deficits in attention span and learning abilities. Adults who drink this water over many years could develop kidney problems or high blood pressure.
Mercury [inorganic]	.002 ppm	1000	2 ppb	2 ppb	Erosion of natural deposits; Discharge from refineries and factories; Runoff from landfills; Runoff from cropland	Some people who drink water containing inorganic mercury well in excess of the MCL over many years could experience kidney damage.
Nitrate		-	10.0 ppm	10.0 ppm	Runoff from fertilizer use; Leaching from septic tanks, sewage; Erosion of natural deposits	Infants below the age of six months who drink water containing nitrate in excess of the MCL could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue baby syndrome.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Nitrite	1.0 ppm	-	1.0 ppm	1.0 ppm	Runoff from fertilizer use; Leaching from septic tanks, sewage; Erosion of natural deposits	Infants below the age of six months who drink water containing nitrite in excess of the MCL could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue baby syndrome.
Selenium	.05 ppm	1000	50 ppb	50 ppb	Discharge from petroleum and metal refineries; Erosion of natural deposits; Discharge from mines	Selenium is an essential nutrient. However, some people who drink water containing selenium in excess of the MCL over many years could experience hair or fingernail losses, numbness in fingers or toes, or problems with their circulation.
Thalium	.002 ppm	1000	2 ppb	0.5 ppb	Leaching from ore-processing sites; Discharge from electronics, glass, and drug factories	Some people who drink water containing thallium in excess of the MCL over many years could experience hair loss, changes in their blood, or problems with their kidneys, intestines, or liver.
Disinfection Byproducts (DBPs)						
Bromate	.010 ppm	1000	10 ppb	0	Byproduct of drinking water disinfection	Some people who drink water containing bromate in excess of the MCL over many years may have an increased risk of getting cancer.
Chloramines	MRDL = 4 ppm	-	MRDL = 4 ppm	MRDLG = 4 ppm	Water additive used to control microbes	Some people who use drinking water containing chloramines well in excess of the MRDL could experience irritating effects to their eyes and nose. Some people who drink water containing chloramines well in excess of the MRDL could experience stomach discomfort or anemia.
Chlorine	MRDL = 4 ppm	-	MRDL = 4 ppm	MRDLG = 4 ppm	Water additive used to control microbes	Some people who use drinking water containing chlorine well in excess of the MRDL could experience irritating effects to their eyes and nose. Some people who drink water containing chlorine well in excess of the MRDL could experience stomach discomfort.
Chlorite	1 ppm	-	1 ppm	0.8 ppm	Byproduct of drinking water disinfection	Some infants and young children who drink water containing chlorite in excess of the MCL could experience nervous system effects. Similar effects may occur in fetuses of pregnant mothers who drink water containing chlorite in excess of the MCL. Some people may experience anemia.
Chlorine dioxide	MRDL = .8 ppm	1000	MRDL = 800 ppb	MRDLG = 800 ppb	Water additive used to control microbes	Some infants and young children who drink water containing chlorine dioxide in excess of the MRDL could experience nervous system effects. Similar effects may occur in fetuses of pregnant mothers who drink water containing chlorine dioxide in excess of the MRDL. Some people may experience anemia.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Haloacetic Acids (HAA5)	60 ppb	-	60 ppb	N/A for combined dichloroacetic acid = 0; monochloroacetic acid = 70 ppb; trichloroacetic acid = 20 ppb	Byproduct of drinking water disinfection	Some people who drink water containing haloacetic acids in excess of the MCL over many years may have an increased risk of getting cancer.
Total Trihalomethanes [TTHMs]	80 ppb	-	80 ppb	N/A (chloroform = 70 ppb; dibromochloromethane = 60 ppb; bromoform = 0; bromodichloromethane = 0)	Byproduct of drinking water disinfection	Some people who drink water containing trihalomethanes in excess of the MCL over many years may experience problems with their liver, kidneys, or central nervous systems, and may have an increased risk of getting cancer.
Synthetic Organic Contaminants including Pesticides and Herbicides, <u>except PFAS</u>						
2,4-D	70 ppb	-	70 ppb	70 ppb	Runoff from herbicide used on row crops	Some people who drink water containing the weed killer 2,4-D well in excess of the MCL over many years could experience problems with their kidneys, liver, or adrenal glands.
2,4,5-TP [Silvex]	50 ppb	-	50 ppb	50 ppb	Residue of banned herbicide	Some people who drink water containing silvex in excess of the MCL over many years could experience liver problems.
Alachlor	2 ppb	-	2 ppb	0	Runoff from herbicide used on row crops	Some people who drink water containing alachlor in excess of the MCL over many years could have problems with their eyes, liver, kidneys, or spleen, or experience anemia, and may have an increased risk of getting cancer.
Atrazine	3 ppb	-	3 ppb	3 ppb	Runoff from herbicide used on row crops	Some people who drink water containing atrazine well in excess of the MCL over many years could experience problems with their cardiovascular system or reproductive difficulties.
Benzo(a)pyrene [PAH]	.2 ppb	1000	200 ppt	0	Leaching from linings of water storage tanks and distribution lines	Some people who drink water containing benzo(a)pyrene in excess of the MCL over many years may experience reproductive difficulties and may have an increased risk of getting cancer.
Carbofuran	40 ppb	-	40 ppb	40 ppb	Leaching of soil fumigant used on rice and alfalfa	Some people who drink water containing carbofuran in excess of the MCL over many years could experience problems with their blood, or nervous or reproductive systems.
Chlordane	2 ppb	-	2 ppb	0	Residue of banned termiticide	Some people who drink water containing chlordane in excess of the MCL over many years could experience problems with their liver or nervous system, and may have an increased risk of getting cancer.
Dalapon	200 ppb	-	200 ppb	200 ppb	Runoff from herbicide used on rights of way	Some people who drink water containing dalapon well in excess of the MCL over many years could experience minor kidney changes.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Di(2-ethylhexyl) adipate	400 ppb	-	400 ppb	400 ppb	Discharge from chemical factories	Some people who drink water containing di (2-ethylhexyl) adipate well in excess of the MCL over many years could experience toxic effects or reproductive difficulties.
Di(2-ethylhexyl) phthalate	6 ppb	-	6 ppb	0	Discharge from rubber and chemical factories	Some people who drink water containing di (2-ethylhexyl) phthalate well in excess of the MCL over many years may have problems with their liver, or experience reproductive difficulties, and may have an increased risk of getting cancer.
Dibromochloropropane [DBCP]	.2 ppb	1000	200 ppt	0	Runoff/ leaching from soil fumigant used on soybeans, cotton, pineapples, and orchards	Some people who drink water containing DBCP in excess of the MCL over many years could experience reproductive problems and may have an increased risk of getting cancer.
Dinoseb	7 ppb	-	7 ppb	7 ppb	Runoff from herbicide used on soybeans and vegetables	Some people who drink water containing dinoseb well in excess of the MCL over many years could experience reproductive difficulties.
Dioxin [2,3,7,8-TCDD]	.03 ppt	1,000	30 ppb	0	Emissions from waste incineration and other combustion; Discharge from chemical factories	Some people who drink water containing dioxin in excess of the MCL over many years could experience reproductive difficulties and may have an increased risk of getting cancer.
Diquat	.02 ppb	1000	20 ppt	20 ppt	Runoff from herbicide use	Some people who drink water containing diquat in excess of the MCL over many years could get cataracts.
Endothall	100 ppb	-	100 ppb	100 ppb	Runoff from herbicide use	Some people who drink water containing endothall in excess of the MCL over many years could experience problems with their stomach or intestines.
Endrin	2 ppb	-	2 ppb	2 ppb	Residue of banned insecticide	Some people who drink water containing endrin in excess of the MCL over many years could experience liver problems.
Ethylene dibromide	.05 ppb	1000	50 ppt	0	Discharge from petroleum refineries	Some people who drink water containing ethylene dibromide in excess of the MCL over many years could experience problems with their liver, stomach, reproductive system, or kidneys, and may have an increased risk of getting cancer.
Glyphosate	700 ppb	-	700 ppb	700 ppb	Runoff from herbicide use	Some people who drink water containing glyphosate in excess of the MCL over many years could experience problems with their kidneys or reproductive difficulties.
Heptachlor	.4 ppb	1000	400 ppt	0	Residue of banned pesticide	Some people who drink water containing heptachlor in excess of the MCL over many years could experience liver damage and may have an increased risk of getting cancer.
Heptachlor epoxide	.2 ppb	1000	200 ppt	0	Breakdown of heptachlor	Some people who drink water containing heptachlor epoxide in excess of the MCL over many years could experience liver damage, and may have an increased risk of getting cancer.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Hexachlorobenzene	1 ppb	-	1 ppb	0	Discharge from metal refineries and agricultural chemical factories	Some people who drink water containing hexachlorobenzene in excess of the MCL over many years could experience problems with their liver or kidneys, or adverse reproductive effects, and may have an increased risk of getting cancer.
Hexachlorocyclo-pentadiene	50 ppb	-	50 ppb	50 ppb	Discharge from chemical factories	Some people who drink water containing hexachlorocyclopentadiene well in excess of the MCL over many years could experience problems with their kidneys or stomach.
Lindane	.2 ppb	1000	200 ppt	200 ppt	Runoff/ leaching from insecticide used on cattle, lumber, gardens	Some people who drink water containing lindane in excess of the MCL over many years could experience problems with their kidneys or liver.
Methoxychlor	40 ppb	-	40 ppb	40 ppb	Runoff/ leaching from insecticide used on fruits, vegetables, alfalfa, livestock	Some people who drink water containing methoxychlor in excess of the MCL over many years could experience reproductive difficulties.
Oxamyl [Vydate]	200 ppb	-	200 ppb	200 ppb	Runoff/ leaching from insecticide used on apples, potatoes and tomatoes	Some people who drink water containing oxamyl in excess of the MCL over many years could experience slight nervous system effects.
PCBs [Polychlorinated biphenyls]	.5 ppb	1000	500 ppt	0	Runoff from landfills; Discharge of waste chemicals	Some people who drink water containing PCBs in excess of the MCL over many years could experience changes in their skin, problems with their thymus gland, immune deficiencies, or reproductive or nervous system difficulties, and may have an increased risk of getting cancer.
Pentachlorophenol	1 ppb	-	1 ppb	0	Discharge from wood preserving factories	Some people who drink water containing pentachlorophenol in excess of the MCL over many years could experience problems with their liver or kidneys, and may have an increased risk of getting cancer.
((~~PFOA~~	~~10 ppt~~	-	~~10 ppt~~	~~N/A~~	~~Run-off or leaching from firefighting foam, industrial discharge, and landfills; wastewater treatment plants~~	~~Some people who drink water containing PFOA in excess of the SAL over many years may experience problems with their cholesterol, liver, thyroid or immune system; have high blood pressure during pregnancy, have babies with lower birthweights; and be at higher risk of getting certain types of cancers.~~
~~PFOS~~	~~15 ppt~~	-	~~15 ppt~~	~~N/A~~	~~Run-off or leaching from firefighting foam, industrial discharge, and landfills; wastewater treatment plants~~	~~Some people who drink water containing PFOS in excess of the SAL over many years may experience problems with their cholesterol, liver, thyroid, kidney, or immune systems; or have children with lower birthweights.~~
~~PFHxS~~	~~65 ppt~~	-	~~65 ppt~~	~~N/A~~	~~Run-off or leaching from firefighting foam, industrial discharge, and landfills; wastewater treatment plants~~	~~Some people who drink water containing PFHxS in excess of the SAL over many years may experience liver or immune problems, or thyroid hormone problems during pregnancy and infancy. It is possible that exposed children may have increased risk of abnormal behavior.~~

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
~~PFNA~~	~~9 ppt~~	~~-~~	~~9 ppt~~	~~N/A~~	~~Run-off or leaching from firefighting foam, industrial discharge, and landfills; wastewater treatment plants~~	~~Some people who drink water containing PFNA in excess of the SAL over many years may experience cholesterol, immune, liver or reproductive problems. Children exposed prenatally may have lower birthweights and increased risk of abnormal development.~~
~~PFBS~~	~~345 ppt~~	~~-~~	~~345 ppt~~	~~N/A~~	~~Run-off or leaching from firefighting foam, industrial discharge, and landfills; wastewater treatment plants~~	~~Some people who drink water containing PFBS in excess of the SAL may experience higher risk of cholesterol, liver, kidney or thyroid problems. Early life is the most sensitive period for altered thyroid hormone; sensitive populations include persons who are pregnant, nursing or less than a year old.~~))
Picloram	500 ppb	-	500 ppb	500 ppb	Herbicide runoff	Some people who drink water containing picloram in excess of the MCL over many years could experience problems with their liver.
Simazine	4 ppb	-	4 ppb	4 ppb	Herbicide runoff	Some people who drink water containing simazine in excess of the MCL over many years could experience problems with their blood.
Toxaphene	3 ppb	-	3 ppb	0	Runoff/ leaching from insecticide used on cotton and cattle	Some people who drink water containing toxaphene in excess of the MCL over many years could have problems with their kidneys, liver, or thyroid, and may have an increased risk of getting cancer.
Synthetic Organic Contaminants - PFAS						
HFPO-DA	10 ppt	-	10 ppt	10 ppt	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing HFPO-DA in excess of the MCL over many years may have increased health risks such as immune, liver, and kidney effects. There is also a potential concern for cancer associated with HFPO-DA exposure. In addition, there may be increased risks of developmental effects for people who drink water containing HFPO-DA in excess of the MCL following repeated exposure during pregnancy and/or childhood.
PFOA	10 ppt (SAL, effective for CCRs until 4/26/27) 4.0 ppt (MCL, effective for CCRs starting 4/26/27)	-	10 ppt (SAL, effective for CCRs until 4/26/27) 4.0 ppt (MCL, effective for CCRs starting 4/26/27)	0 ppt	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing PFOA in excess of the MCL over many years may have increased health risks such as cardiovascular, immune, and liver effects, as well as increased incidence of certain types of cancers including kidney and testicular cancer. In addition, there may be increased risks of developmental and immune effects for people who drink water containing PFOA in excess of the MCL following repeated exposure during pregnancy and/or childhood.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
PFOS	15 ppt (SAL, effective for CCRs until 4/26/27) 4.0 ppt (MCL, effective for CCRs starting 4/26/27)	-	15 ppt (SAL, effective for CCRs until 4/26/27) 4.0 ppt (MCL, effective for CCRs starting 4/26/27)	0 ppt	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing PFOS in excess of the MCL over many years may have increased health risks such as cardiovascular, immune, and liver effects, as well as increased incidence of certain types of cancers including liver cancer. In addition, there may be increased risks of developmental and immune effects for people who drink water containing PFOS in excess of the MCL following repeated exposure during pregnancy and/or childhood.
PFHxS	65 ppt (SAL, effective for CCRs until 4/26/27) 10 ppt (MCL, effective for CCRs starting 4/26/27)	-	65 ppt (SAL, effective for CCRs until 4/26/27) 10 ppt (MCL, effective for CCRs starting 4/26/27)	10 ppt	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing PFHxS in excess of the MCL over many years may have increased health risks such as immune, thyroid, and liver effects. In addition there may be increased risks of developmental effects for people who drink water containing PFHxS in excess of the MCL following repeated exposure during pregnancy and/or childhood.
PFNA	9 ppt (SAL, effective for CCRs until 4/26/27) 10 ppt (MCL, effective for CCRs starting 4/26/27)	-	9 ppt (SAL, effective for CCRs until 4/26/27) 10 ppt (MCL, effective for CCRs starting 4/26/27)	10 ppt	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing PFNA in excess of the MCL over many years may have increased health risks such as elevated cholesterol levels, immune effects, and liver effects. In addition, there may be increased risks of developmental effects for people who drink water containing PFNA in excess of the MCL following repeated exposure during pregnancy and/or childhood.
PFBS	345 ppt (SAL, effective for CCRs until 4/26/27) MCL is part of Hazard Index as of 4/26/27)	-	345 ppt (SAL, effective for CCRs until 4/26/27) MCL is part of Hazard Index as of 4/26/27)	-	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Some people who drink water containing PFBS in excess of the SAL may experience higher risk of cholesterol, liver, kidney or thyroid problems. Early life is the most sensitive period for altered thyroid hormone; sensitive populations include persons who are pregnant, nursing or less than a year old.
Hazard Index PFAS (HFPO-DA, PFBS, PFHxS, and PFNA)	1 (unitless)	-	1 (unitless)	1 (unitless)	Discharge from manufacturing and industrial chemical facilities, use of certain consumer products, occupational exposures, and certain firefighting activities	Per- and polyfluoroalkyl substances (PFAS) can persist in the human body and exposure may lead to increased risk of adverse health effects. Low levels of multiple PFAS that individually would not likely result in increased risk of adverse health effects may result in adverse health effects when combined in a mixture. Some people who consume drinking water containing mixtures of PFAS in excess of the Hazard Index (HI) MCL may have increased health risks such as liver, immune, and thyroid effects following exposure over many years and developmental and thyroid effects following repeated exposure during pregnancy and/or childhood.
Volatile Organic Contaminants						

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Benzene	5 ppb	-	5 ppb	0	Discharge from factories; Leaching from gas storage tanks and landfills	Some people who drink water containing benzene in excess of the MCL over many years could experience anemia or a decrease in blood platelets, and may have an increased risk of getting cancer.
Carbon tetrachloride	5 ppb	-	5 ppb	0	Discharge from chemical plants and other industrial activities	Some people who drink water containing carbon tetrachloride in excess of the MCL over many years could experience problems with their liver and may have an increased risk of getting cancer.
Chlorobenzene	100 ppb	-	100 ppb	100 ppb	Discharge from chemical and agricultural chemical factories	Some people who drink water containing chlorobenzene in excess of the MCL over many years could experience problems with their liver or kidneys.
o-Dichlorobenzene	600 ppb	-	600 ppb	600 ppb	Discharge from industrial chemical factories	Some people who drink water containing o-dichlorobenzene well in excess of the MCL over many years could experience problems with their liver, kidneys, or circulatory systems.
p-Dichlorobenzene	75 ppb	-	75 ppb	75 ppb	Discharge from industrial chemical factories	Some people who drink water containing p-dichlorobenzene in excess of the MCL over many years could experience anemia, damage to their liver, kidneys, or spleen, or changes in their blood.
1,2-Dichloroethane	5 ppb	-	5 ppb	0	Discharge from industrial chemical factories	Some people who drink water containing 1,2-dichloroethane in excess of the MCL over many years may have an increased risk of getting cancer.
1,1-Dichloroethylene	7 ppb	-	7 ppb	7 ppb	Discharge from industrial chemical factories	Some people who drink water containing 1,1-dichloroethylene in excess of the MCL over many years could experience problems with their liver.
cis-1,2-Dichloroethylene	70 ppb	-	70 ppb	70 ppb	Discharge from industrial chemical factories	Some people who drink water containing cis-1,2-dichloroethylene in excess of the MCL over many years could experience problems with their liver.
trans-1,2-Dichloroethylene	100 ppb	-	100 ppb	100 ppb	Discharge from industrial chemical factories	Some people who drink water containing trans-1,2-dichloroethylene well in excess of the MCL over many years could experience problems with their liver.
Dichloromethane	5 ppb	-	5 ppb	0	Discharge from pharmaceutical and chemical factories	Some people who drink water containing dichloromethane in excess of the MCL over many years could have liver problems and may have an increased risk of getting cancer.
1,2-Dichloropropane	5 ppb	-	5 ppb	0	Discharge from industrial chemical factories	Some people who drink water containing 1,2-dichloropropane in excess of the MCL over many years may have an increased risk of getting cancer.
Ethylbenzene	700 ppb	-	700 ppb	700 ppb	Discharge from petroleum refineries	Some people who drink water containing ethylbenzene well in excess of the MCL over many years could experience problems with their liver or kidneys.
Styrene	100 ppb	-	100 ppb	100 ppb	Discharge from rubber and plastic factories; Leaching from landfills	Some people who drink water containing styrene well in excess of the MCL over many years could have problems with their liver, kidneys, or circulatory system.

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
Tetrachloroethylene	5 ppb	-	5 ppb	0	Discharge from factories and dry cleaners	Some people who drink water containing tetrachloroethylene in excess of the MCL over many years could have problems with their liver, and may have an increased risk of getting cancer.
1,2,4-Trichlorobenzene	70 ppb	-	70 ppb	70 ppb	Discharge from textile-finishing factories	Some people who drink water containing 1,2,4-trichlorobenzene well in excess of the MCL over many years could experience changes in their adrenal glands.
1,1,1-Trichloroethane	200 ppb	-	200 ppb	200 ppb	Discharge from metal degreasing sites and other factories	Some people who drink water containing 1,1,1-trichloroethane in excess of the MCL over many years could experience problems with their liver, nervous system, or circulatory system.
1,1,2-Trichloroethane	5 ppb	-	5 ppb	3 ppb	Discharge from industrial chemical factories	Some people who drink water containing 1,1,2-trichloroethane well in excess of the MCL over many years could have problems with their liver, kidneys, or immune systems.
Toluene	1000 ppm	-	1000 ppm	1000 ppm	Discharge from petroleum factories	Some people who drink water containing toluene well in excess of the MCL over many years could have problems with their nervous system, kidneys, or liver.
Vinyl Chloride	2 ppb	-	2 ppb	0	Leaching from PVC piping: Discharge from plastics factories	Some people who drink water containing vinyl chloride in excess of the MCL over many years may have an increased risk of getting cancer.
Xylenes	10,000 ppb	-	10,000 ppb	10,000 ppb	Discharge from petroleum factories; Discharge from chemical factories	Some people who drink water containing xylenes in excess of the MCL over many years could experience damage to their nervous system.
Treatment Technique Violations						
Acrylamide	TT	-	TT	0	Added to water during sewage/ wastewater treatment	Some people who drink water containing high levels of acrylamide over a long period of time could have problems with their nervous system or blood, and may have an increased risk of getting cancer.
Epichlorohydrin	TT	-	TT	0	Discharge from industrial chemical factories; an impurity of some water treatment chemicals	Some people who drink water containing high levels of epichlorohydrin over a long period of time could experience stomach problems, and may have an increased risk of getting cancer.
Groundwater rule TT violations	TT	-	TT	N/A	-	Inadequately treated or inadequately protected water may contain disease-causing organisms. These organisms can cause symptoms such as diarrhea, nausea, cramps, and associated headaches.

Key

AL = Action Level

MCL = Maximum Contaminant Level

MCLG = Maximum Contaminant Level Goal

MFL = million fibers per liter

MRDL = Maximum Residual Disinfectant Level

MRDLG = Maximum Residual Disinfectant Level Goal

mrem/year = millirems per year (a measure of radiation absorbed by the body)

N/A = Not Applicable

Contaminant	MCL or SAL (units match lab results)	To convert lab results for CCR, multiply by	MCL or SAL in CCR units	MCLG in CCR units	Major Sources in Drinking Water	Health Effects Language
NTU = Nephelometric Turbidity Units (a measure of water clarity) **pCi/L** = picocuries per liter (a measure of radioactivity) **ppm** = parts per million, or milligrams per liter (mg/L) **ppb** = parts per billion, or micrograms per liter (ug/L) **ppt** = parts per trillion, or nanograms per liter (ng/L) **ppq** = parts per quadrillion, or picograms per liter (pg/L) **SAL** = state action level **TT** = Treatment Technique						

AMENDATORY SECTION (Amending WSR 21-23-096, filed 11/17/21, effective 1/1/22)

WAC 246-390-010 Definitions, abbreviations, and acronyms. The definitions, abbreviations, and acronyms in this section apply throughout this chapter, unless the context clearly indicates otherwise.

(1) "µmhos/cm" means micromhos per centimeter (1 µmhos/cm = 1 S/cm).

(2) "µg/L" means micrograms per liter (1 µg/L = 1 ppb).

(3) "Acute" means posing an immediate risk to human health.

(4) "Bioaccumulative" means a chemical that can accumulate in the body when regular exposure occurs through drinking water.

(5) "C.F.R." means the Code of Federal Regulations.

(6) "CFU" means colony-forming unit.

(7) "Chronic" means posing a risk to human health only when exposure occurs over many years to a contaminant above a state or federal health standard.

(8) "Close of business" means the latest time during a business day when a lab is no longer in routine operation for accepting or performing drinking water sample analysis.

(9) "Confirmation" means to demonstrate that the results of a sample accurately represents the original sample result by analyzing another sample from the same location within a reasonable given period of time. This confirmation analysis is in addition to any analytical method confirmation requirements.

(10) "Contaminant" means a substance present in drinking water that may adversely affect the health of the consumer or the aesthetic quality of the water. It is measured using an analytical method for compliance purposes under chapters 246-290 and 246-291 WAC.

(11) "Contracted lab" means a certified lab that receives a drinking water sample from another certified lab for analysis.

(12) "Contracting lab" means a certified lab that sends a drinking water sample to another certified lab to be analyzed.

(13) "CU" means color unit.

(14) "Department" means the Washington state department of health or health officer as identified in a joint plan of responsibility under WAC 246-290-030(1).

(15) "Ecology" means the Washington state department of ecology.

(16) "EPA" means the United States Environmental Protection Agency.

(17) "Estimated concentration" means the level of the contaminant reported to the department is above a lab's MDL, but below the lab's MRL.

(18) "GWR" means groundwater rule.

(19) "Lab" or "certified lab" means an environmental lab accredited under chapter 173-50 WAC for one or more drinking water contaminants and meets the requirements of this chapter.

(20) "Maximum contaminant level (MCL)" means the maximum permissible level of a contaminant in water that a public water system delivers to consumers. MCLs are established in chapters 246-290 and 246-291 WAC.

(21) "Minimum detectable activity (MDA)" means the smallest activity or concentration of radioactive material in a sample that will yield a net count (above sample background) that can be detected with 95 percent probability.

(22) "Minimum detection level (MDL)" means the minimum measured concentration of a substance that can be reported with 99 percent confidence that the measured concentration is distinguishable from the method blank results.

(23) "Method reporting limit (MRL)" means the lowest concentration of a standard used for calibration.

(24) "MFL" means microfibers per liter.

(25) "mg/L" means milligrams per liter (1 mg/L = 1 ppm).

(26) "MPN" means most probable number.

(27) "ng/L" means nanograms per liter (1 ng/L = 1 ppt).

(28) "NTU" means nephelometric turbidity units.

(29) "pCi/L" means picocuries per liter.

(30) "ppb" means parts per billion (1 ppb = 1 µg/L).

(31) "ppm" means parts per million (1 ppm = 1 mg/L).

(32) "ppt" means parts per trillion (1 ppt = 1 ng/L).

(33) "Proficiency testing (PT)" means the evaluation of sample analysis results, the true values of which are known to the supplier of the samples, but unknown to the lab conducting the analysis. PT samples are provided by a source external to the certified lab.

(34) "Public water system" is defined under WAC 246-290-020 and 246-291-010.

(35) "Quality control (QC)" means a set of measures used during an analytical method to ensure that the process is within specified control parameters.

(36) "State action level (SAL)" means the concentration of a contaminant or group of contaminants((~~, without an MCL,~~)) established to protect public health in accordance with WAC 246-290-315 and which, if exceeded, triggers actions a purveyor takes in accordance with WAC 246-290-320.

(37) "State detection reporting limit (SDRL)" means the minimum reportable detection of a contaminant as established in Tables 3 through 7 of this chapter.

(38) "Tentatively identified compound (TIC)" means compounds detected in samples that are not target compounds, internal standards, system monitoring compounds or surrogates.

AMENDATORY SECTION (Amending WSR 21-23-096, filed 11/17/21, effective 1/1/22)

WAC 246-390-075 Reporting. (1) A lab shall report analytical results to the department and the public water system.

(2) A lab submitting paper reports shall complete and submit to the department data reports following the procedures and templates in the department's *Laboratory Reporting Guidance*, Publication DOH 331-530, ((~~January 2022~~)) April 2025.

(3) A lab submitting electronic reports shall complete and submit to the department data reports following the procedures in the department's *Electronic Reporting Guidance*, Publication 331-289, ((~~January 2022~~)) April 2025.

(4) Labs shall submit reports to the public water system in the format and time frame that was agreed upon when executing the service agreement between the laboratory and the public water system.

(5) Labs shall submit reports of acute contaminant results within 10 business days after receiving the sample.

(6) Labs shall submit reports of chronic contaminants within 30 calendar days after receiving the sample.

(7) Analytical results must be complete, legible, and accurate.

(8) A lab shall report numerical results consistent with the accuracy of the EPA-approved methods and any associated lab instruments, glassware, or tools.

(9) A lab shall report numerical results out to, but not exceed, one decimal place past the SDRL in cases where the last definitely known digit exceeds one decimal place past the SDRL as follows:

(a) If the digit 5, 6, 7, 8, or 9 is dropped, increase the preceding digit by one unit;

(b) If the digit 0, 1, 2, 3, or 4 is dropped, do not alter the preceding digit; or

(c) If the SDRL is 1.1 and the result, out to the last definitely known digit is ((~~1.132~~)) 1.13, then the value reported to the department is ((~~1.13;~~

~~(b) If the digit 6, 7, 8, or 9 is dropped, increase the preceding digit by one unit;~~

~~(c) If the digit 0, 1, 2, 3, or 4 is dropped, do not alter the preceding digit; or~~

~~(d) If the digit 5 is dropped, round off the preceding digit to the nearest even number. For example, 2.25 becomes 2.2, and 2.35 becomes 2.4~~)) 1.1. If the result is 1.15, then the value reported to the department is 1.2.

(10) A lab shall include the following data qualifiers adjacent to the results that are affected:

(a) "B" - This data qualifier is used when the target contaminant is detected in the method blank above the lab's established MRL or SDRL, whichever is lower;

(b) "J" - This data qualifier is used when the result is an estimated concentration per subsections (13), (14), and (17) of this section;

(c) "NDDS" - This data qualifier is used when the contaminant is not detected in duplicate sample; or

(d) "U" - This data qualifier is used when the radiochemistry contaminant is not detected at or above the lab's established MDA.

(11) A lab shall notate on the report to the public water system and the department when any analysis is completed using a provisional accreditation.

(12) At the department's request, a lab shall submit the following information:

(a) The method specific QC for any given analytical report.

(b) The most recent MDL procedures performed for any given contaminant.

(c) The most recent PT study performed for any given contaminant.

(13) The SDRLs for organic chemical contaminants are established in Tables 3 and 7 of this section. All contaminants in Tables 3 and 7 are considered chronic contaminants.

(a) Labs shall ((~~attach to the analytical result a copy of the method specific QC results for any organic chemical detection that is reported to the department which is at or above the SDRLs listed in Table 3 of this section except for:~~

~~(i) Chloroform (0027);~~

~~(ii) Bromodichloromethane (0028);~~

~~(iii) Dibromochloromethane (0029);~~

~~(iv) Bromoform (0030);~~

~~(v) Monochloroacetic Acid (0411);~~

~~(vi) Dichloroacetic Acid (0412);~~
~~(vii) Trichloroacetic Acid (0413);~~
~~(viii) Monobromoacetic Acid (0414);~~
~~(ix) Monobromoacetic Acid (0415); and~~
~~(x) Total Organic Carbon (0421)~~)) provide a copy of the method specific QC results at the request of the department.

(b) A lab shall report organic chemical contaminant results when the lab's established MRL is greater than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL;

(ii) An estimated concentration, notated with a "J" data qualifier when a result is equal to or greater than the SDRL, but less than the lab's established MRL;

(iii) A number when a result is equal to or greater than the lab's established MRL.

(c) A lab shall report organic chemical contaminant results when the lab's established MRL is less than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the lab's established MRL;

(ii) "Nondetect" or "ND" when a lab's result is less than the established SDRL; or

(iii) A number when a result is equal to or greater than the SDRL.

(d) A lab shall report organic chemical contaminant results when their established MRL is equal to the SDRL as:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL; or

(ii) A number when a result is equal to or greater than the SDRL and the lab's established MRL.

Table 3 - Organic Contaminants

Contaminant Name	Contaminant Number	Units	SDRL
1,1 Dichloroethane	0058	µg/L	0.5
1,1 Dichloroethylene	0046	µg/L	0.5
1,1 Dichloropropene	0062	µg/L	0.5
1,1,1 Trichloroethane	0047	µg/L	0.5
1,1,1,2 Tetrachloroethane	0072	µg/L	0.5
1,1,2 Trichloroethane	0067	µg/L	0.5
1,1,2,2 Tetrachloroethane	0080	µg/L	0.5
1,2 Dichlorobenzene	0084	µg/L	0.5
1,2 Dichloroethane	0050	µg/L	0.5
1,2 Dichloropropane	0063	µg/L	0.5
1,2,3 Trichlorobenzene	0098	µg/L	0.5
1,2,3 Trichloropropane	0079	µg/L	0.5
1,2,4 Trichlorobenzene	0095	µg/L	0.5
1,2,4 Trimethylbenzene	0091	µg/L	0.5
1,3 Dichloropropane	0070	µg/L	0.5
1,3 Dichloropropene	0154	µg/L	0.5
1,3,5 Trimethylbenzene	0089	µg/L	0.5
1,4 Dichlorobenzene	0052	µg/L	0.5
2,2 Dichloropropane	0059	µg/L	0.5
2,3,7,8 TCDD (dioxin)	0149	ng/L	0.005

Contaminant Name	Contaminant Number	Units	SDRL
2,4 D	0037	µg/L	0.1
2,4 DB	0135	µg/L	1
2,4,5 T	0136	µg/L	0.4
2,4,5 TP (Silvex)	0038	µg/L	0.2
3,5 Dichlorbenzoic Acid	0226	µg/L	0.5
4,4 DDD	0232	µg/L	0.1
4,4 DDE	0233	µg/L	0.1
4,4 DDT	0234	µg/L	0.1
Acenaphthylene	0244	µg/L	0.2
Acifluorfen	0223	µg/L	2
Alachlor	0117	µg/L	0.2
Aldicarb	0142	µg/L	0.5
Aldicarb Sulfone	0143	µg/L	0.8
Aldicarb Sulfoxide	0144	µg/L	0.5
Aldrin	0118	µg/L	0.1
Anthracene	0246	µg/L	0.2
Arochlor 1016	0180	µg/L	0.08
Arochlor 1221	0173	µg/L	20
Arochlor 1232	0174	µg/L	0.5
Arochlor 1242	0175	µg/L	0.3
Arochlor 1248	0176	µg/L	0.1
Arochlor 1254	0177	µg/L	0.1
Arochlor 1260	0178	µg/L	0.2
Atrazine	0119	µg/L	0.1
Bentazon	0220	µg/L	0.5
Benzene	0049	µg/L	0.5
Benzo (a) anthracene	0247	µg/L	0.2
Benzo (a) Pyrene	0120	µg/L	0.02
Benzo (b) fluoroanthene	0248	µg/L	0.2
Benzo (k) fluoranthene	0250	µg/L	0.2
Benzyl Butyl Phthalate	0258	µg/L	1.0
Bromacil	0179	µg/L	0.1
Bromobenzene	0078	µg/L	0.5
Bromochloromethane	0086	µg/L	0.5
Bromodichloromethane	0028	µg/L	0.5
Bromoform	0030	µg/L	0.5
Bromomethane	0054	µg/L	0.5
Butachlor	0121	µg/L	0.1
Carbaryl	0145	µg/L	2
Carbofuran	0146	µg/L	0.9
Carbon Tetrachloride	0048	µg/L	0.5
Chlordane (total)	0122	µg/L	0.2
Chlorobenzene	0071	µg/L	0.5
Chloroethane	0055	µg/L	0.5
Chloroform	0027	µg/L	0.5
Chloromethane	0053	µg/L	0.5

Contaminant Name	Contaminant Number	Units	SDRL
Chrysene	0251	µg/L	0.2
Cis- 1,2 Dichloroethylene	0060	µg/L	0.5
Cis- 1,3 Dichloropropene	0065	µg/L	0.5
Dalapon	0137	µg/L	1
DBCP	0103	µg/L	0.02
DBCP (screening)	0428	µg/L	0.5
DCPA Acid Metabolites	0225	µg/L	0.1
Di (2-Ethylhexyl) Adipate	0124	µg/L	0.6
Di (2-Ethylhexyl) Phthalate	0125	µg/L	0.6
Dibromoacetic Acid	0415	µg/L	1
Dibromochloromethane	0029	µg/L	0.5
Dibromomethane	0064	µg/L	0.5
Dicamba	0138	µg/L	0.2
Dichloroacetic Acid	0412	µg/L	1
Dichlorodifluoromethane	0104	µg/L	0.5
Dichlorprop	0221	µg/L	0.5
Dieldrin	0123	µg/L	0.1
Diethyl Phthalate	0260	µg/L	1.0
Dimethyl Phthalate	0261	µg/L	1.0
Di-n-butyl Phthalate	0259	µg/L	1.0
Dinoseb	0139	µg/L	0.2
Diquat	0150	µg/L	0.4
EDB	0102	µg/L	0.01
EDB (screening)	0427	µg/L	0.5
Endothal	0151	µg/L	9
Endrin	0033	µg/L	0.01
EPTC	0208	µg/L	0.1
Ethylbenzene	0073	µg/L	0.5
Fluorene	0254	µg/L	0.2
Glyphosate	0152	µg/L	6
HAA(5)	0416	µg/L	+
Heptachlor	0126	µg/L	0.04
Heptachlor Epoxide	0127	µg/L	0.02
Hexachlorobenzene	0128	µg/L	0.1
Hexachlorobutadiene	0097	µg/L	0.5
Hexachlorocyclo pentadiene	0129	µg/L	0.1
Isopropylbenzene	0087	µg/L	0.5
Lindane (bhc - gamma)	0034	µg/L	0.02
M- dichlorobenzene	0083	µg/L	0.5
M/P Xylenes (MCL for total)	0074	µg/L	0.5
Methomyl	0147	µg/L	4
Methoxychlor	0035	µg/L	0.1
Methylene Chloride (Dichloromethane)	0056	µg/L	0.5
Metolachlor	0130	µg/L	0.1
Metribuzin	0131	µg/L	0.1
Molinate	0218	µg/L	0.1

Contaminant Name	Contaminant Number	Units	SDRL
Monobromoacetic Acid	0414	µg/L	1
Monochloroacetic Acid	0411	µg/L	2
Naphthalene	0096	µg/L	0.5
N-Butylbenzene	0094	µg/L	0.5
N-Propylbenzene	0088	µg/L	0.5
O- Chlorotoluene	0081	µg/L	0.5
O- Xylene (MCL for total)	0075	µg/L	0.5
Oxamyl	0148	µg/L	2
P- Chlorotoluene	0082	µg/L	0.5
Paraquat	0400	µg/L	0.8
PCB (as Decachlorobiphenyl)	0401	µg/L	0.1
Pentachlorophenol	0134	µg/L	0.04
Phenanthrene	0256	µg/L	0.2
Picloram	0140	µg/L	0.1
P-Isopropyltoluene	0093	µg/L	0.5
Propachlor	0132	µg/L	0.1
Pyrene	0257	µg/L	0.2
Sec- Butylbenzene	0092	µg/L	0.5
Simazine	0133	µg/L	0.07
Styrene	0076	µg/L	0.5
Terbacil	0190	µg/L	0.1
Tert- Butylbenzene	0090	µg/L	0.5
Tetrachloroethylene	0068	µg/L	0.5
Toluene	0066	µg/L	0.5
Total organic carbon	0421	mg/L	0.7
Total Trihalomethane	0031	µg/L	+
Total Xylenes	0160	µg/L	0.5
Toxaphene	0036	µg/L	1
Trans- 1,2 Dichloroethylene	0057	µg/L	0.5
Trans- 1,3 Dichloropropene	0069	µg/L	0.5
Trichloroacetic Acid	0413	µg/L	1
Trichloroethylene	0051	µg/L	0.5
Trichlorofluoromethane	0085	µg/L	0.5
Trifluralin	0243	µg/L	0.1
Vinyl Chloride	0045	µg/L	0.5

+ Results are calculated values based on other analytical results.

(14) The SDRLs for inorganic chemical contaminants are established in Table 4 of this section. All contaminants in Table 4 are considered chronic contaminants except annual, quarterly, or monthly nitrate analysis which is considered an acute contaminant. Labs shall report analytical results within 10 business days after receiving the nitrate sample. If nitrate analysis is part of a routine complete inorganic compound panel, then labs shall submit a report to the department within 30 calendar days after receiving the sample.

(a) Labs shall provide a copy of the method specific QC results at the request of the department.

(b) A lab shall report inorganic chemical contaminant results when the lab's established MRL is greater than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL;

(ii) An estimated concentration, notated with a "J" data qualifier, when a result is equal to or greater than the SDRL, but less than the lab's established MRL; or

(iii) A number when a result is equal to or greater than the lab's established MRL.

((~~(b)~~)) (c) A lab shall report inorganic chemical contaminant results when the lab's established MRL is less than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the lab's established MRL;

(ii) "Nondetect" or "ND" when a lab's result is less than the department's established SDRL, but greater than the lab's established MRL; or

(iii) A number when a result is equal to or greater than the SDRL.

((~~(c)~~)) (d) A lab shall report inorganic chemical contaminant results when the lab's established MRL is equal to the SDRL as:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL; or

(ii) A number when a result is equal to or greater than the SDRL and the lab's established MRL.

Table 4 - Inorganic Contaminants

Contaminant Name	Contaminant Number	Units	SDRL
Alkalinity-Lab	0403	mg/L	5
Antimony	0112	mg/L	0.003
Arsenic	0004	mg/L	0.001
Asbestos	0115	MFL	0.20
Barium	0005	mg/L	0.1
Beryllium	0110	mg/L	0.0003
Bromate	0419	mg/L	0.005/0.001
Cadmium	0006	mg/L	0.001
Chloride	0021	mg/L	2
Chlorite	0418	mg/L	0.02
Chromium	0007	mg/L	0.007
Color	0018	CU	15
Conductivity	0016	µmhos/cm	70
Copper	0023	mg/L	0.02
Cyanide	0116	mg/L	0.05
Fluoride	0019	mg/L	0.2
Hardness	0015	mg/L	10
Iron	0008	mg/L	0.1
Lead	0009	mg/L	0.001
Manganese	0010	mg/L	0.01
Mercury	0011	mg/L	0.0002
Nickel	0111	mg/L	0.005
Nitrate-n	0020	mg/L	0.5
Nitrite-n	0114	mg/L	0.1
Selenium	0012	mg/L	0.002
Silver	0013	mg/L	0.1

Contaminant Name	Contaminant Number	Units	SDRL
Sodium	0014	mg/L	5
Sulfate	0022	mg/L	2
TDS-total dissolved solids	0026	mg/L	100
Thallium	0113	mg/L	0.001
Total nitrate/nitrite	0161	mg/L	0.5
Turbidity	0017	NTU	0.1
Zinc	0024	mg/L	0.2

Labs that use EPA Methods 317.0, 326.0 or 321.8 must meet a 0.0010 mg/L SDRL for bromate.

(15) The SDRLs for radiochemistry contaminants are established in Table 5 of this section. All contaminants in Table 5 are considered chronic contaminants.

(a) A lab's MDA must meet the established SDRL levels for the analysis to be considered for compliance purposes.

(b) A lab shall report radiochemistry contaminant results as:

(i) A number and a "U" qualifier if the contaminant was analyzed for, but not detected at or above the lab's established MDA; or

(ii) A number when a result is equal to or greater than the lab's established MDA.

Table 5 - Radiochemistry Contaminants

Contaminant Name	Contaminant Number	Units	SDRL
Cesium 134	0107	pCi/L	10.0
Gross Alpha	0165	pCi/L	3.0
Gross Alpha (Minus Uranium)	0041	pCi/L	+
Gross Beta	0042	pCi/L	4.0
Iodine 131	0108	pCi/L	1.0
Radium 226	0039	pCi/L	1.0
Radium 226 + 228	0040	pCi/L	+
Radium 228	0166	pCi/L	1.0
Radon	0109	pCi/L	+
Strontium 90	0044	pCi/L	2.0
Tritium	0043	pCi/L	1000
Uranium	0105	µg/L	1.0

+ Results are calculated values based on other analytical results.

(16) The units for microbiology contaminants are established in Table 6 of this section. All contaminants in Table 6 are considered acute contaminants.

(a) Total coliform and *E. coli* results for routine and repeat samples in accordance with 40 C.F.R. 141 Subpart Y - Revised Total Coliform Rule, GWR triggered, and GWR assessment source sample results that are absent or present as follows:

(i) "Satisfactory" if no total coliforms are detected.

(ii) "Unsatisfactory" if:

(A) Total coliforms are detected; and

(B) *E. coli* absent if *E. coli* is not detected; or

(C) *E. coli* present if *E. coli* is detected.

(b) A lab shall report routine filtered and unfiltered surface water microbiology contaminant results as a number.

(c) A lab shall report routine heterotrophic plate count results as a number.

(d) A lab shall report results of investigative samples or samples collected for information only to the public water system for total coliforms, fecal coliforms, and *E. coli* as a number or, as absent or present. Investigative samples or samples collected for information only are not required to be reported to the department.

Table 6 - Microbiology Contaminants

Contaminant Name	Contaminant Number	Units
E. coli (numerical)	0003	CFU/100mL
E. coli (numerical)	0003	MPN/100mL
E. coli (absence/presence)	0003	N/A
Fecal Coliform (numerical)	0002	CFU/100mL
Fecal Coliform (numerical)	0002	MPN/100mL
Fecal Coliform (absence/presence)	0002	N/A
Heterotrophic Plate Count (numerical)	0101	CFU/1mL
Heterotrophic Plate Count (numerical)	0101	MPN/mL
Total Coliform (numerical)	0001	CFU/100mL
Total Coliform (numerical)	0001	MPN/100mL
Total Coliform (absence/presence)	0001	N/A

(17) The SDRLs for per- and polyfluoroalkyl substances (PFAS) are established in Table 7 of this section. All contaminants in Table 7 are considered chronic contaminants.

(a) A lab shall analyze PFAS samples using EPA method 537.1 version 2 (3/20), or EPA method 533((~~, or with written approval, other department-approved methods~~)).

(b) Labs shall provide a copy of the method specific QC results at the request of the department.

(c) A lab shall report PFAS contaminant results when the lab's established MRL is greater than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL;

(ii) An estimated concentration, notated with a "J" data qualifier when a result is equal to or greater than the SDRL, but less than the lab's established MRL; or

(iii) A number when a result is equal to or greater than the lab's established MRL.

((~~(c)~~)) (d) A lab shall report PFAS contaminant results when the lab's established MRL is less than the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the lab's established MRL;

(ii) "Nondetect" or "ND" when a lab's result is less than the established SDRL; or

(iii) A number when a result is equal to or greater than the SDRL.

((~~(d)~~)) (e) A lab shall report PFAS contaminant results when the lab's established MRL is equal to the SDRL as follows:

(i) "Nondetect" or "ND" when a lab's result is less than the SDRL and MRL; or

(ii) A number when a result is equal to or greater than the SDRL and the lab's established MRL.

((~~(e)~~)) (f) A lab shall report to the department any tentatively identified compounds (TIC) that are detected while analyzing a PFAS sample if the approved method allows for TIC determinations to be made.

((~~(f)~~)) (g) A lab shall ((~~attach to the analytical result~~)) provide a copy of the method-specific QC results for any TIC detections that are reported to the department.

Table 7 - Per- and Polyfluoroalkyl Contaminants

Contaminant Name	Contaminant Number	Units	SDRL	[3]Required Contaminant List for EPA 537.1 version 2 (3/20)	[4]Required Contaminant List for EPA 533
(11Cl-PF3OUdS) 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	448	ng/L	2.0	Y	Y
(4:2FTS) 1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	450	ng/L	2.0	N	Y
(6:2FTS) 1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	451	ng/L	2.0	N	Y
(8:2FTS) 1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	452	ng/L	2.0	N	Y
(9Cl-PF3ONS) 9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	446	ng/L	2.0	Y	Y
(ADONA) 4,8-Dioxa-3H-perfluorononanoic acid	445	ng/L	2.0	Y	Y
(HFPO-DA) Hexafluoropropylene oxide dimer acid	447	ng/L	2.0	Y	Y
(NEtFOSAA) N-ethyl perfluorooctanesulfonamidoacetic acid	441	ng/L	((~~3~~)) 2.0	Y	N
(NFDHA) Nonafluoro-3,6-dioxaheptanoic acid	453	ng/L	2.0	N	Y
(NMeFOSAA) N-methyl perfluorooctanesulfonamidoacetic acid	442	ng/L	((~~3~~)) 2.0	Y	N
(PFBA) Perfluorobutanoic acid	454	ng/L	2.0	N	Y
(PFBS) Perfluorobutanesulfonic acid	429	ng/L	2.0	Y	Y
(PFDA) Perfluorodecanoic acid	436	ng/L	2.0	Y	Y
(PFDoA) Perfluorododecanoic acid	438	ng/L	2.0	Y	Y
(PFEESA) Perfluoro(2-ethoxyethane)sulfonic acid	460	ng/L	2.0	N	Y
(PFHpA) Perfluoroheptanoic acid	430	ng/L	2.0	Y	Y
(PFHpS) Perfluoroheptanesulfonic acid	455	ng/L	2.0	N	Y
(PFHxA) Perfluorohexanoic acid	435	ng/L	2.0	Y	Y
(PFHxS) Perfluorohexanesulfonic acid	431	ng/L	2.0	Y	Y
(PFMBA) Perfluoro-4-methoxybutanoic acid	456	ng/L	2.0	N	Y
(PFMPA) Perfluoro-3-methoxypropanoic acid	457	ng/L	2.0	N	Y
(PFNA) Perfluorononanoic acid	432	ng/L	2.0	Y	Y
(PFOA) Perfluorooctanoic acid	434	ng/L	2.0	Y	Y
(PFOS) Perfluorooctanesulfonic acid	433	ng/L	2.0	Y	Y
(PFPeA) Perfluoropentanoic acid	458	ng/L	2.0	N	Y
(PFPeS) Perfluoropentanesulfonic acid	459	ng/L	2.0	N	Y
(PFTA) Perfluorotetradecanoic acid	440	ng/L	2.0	Y	N
(PFTrDA) Perfluorotridecanoic acid	439	ng/L	2.0	Y	N
(PFUnA) Perfluoroundecanoic acid	437	ng/L	2.0	Y	Y

[3] For a water system to qualify for a monitoring waiver these contaminants must be reported to the department if analyzing the sample using EPA method 537.1 version 2 (3/20).

[4] For a water system to qualify for a monitoring waiver these contaminants must be reported to the department if analyzing the sample using EPA method 533.

RULE-MAKING ORDER
PERMANENT RULE ONLY

CR-103P (December 2017)
(Implements RCW 34.05.360)

CODE REVISER USE ONLY

OFFICE OF THE CODE REVISER
STATE OF WASHINGTON
FILED

DATE: July 15, 2025
TIME: 8:54 AM

WSR 25-15-072

Agency: Washington State Board of Health

Effective date of rule:

Permanent Rules

☒ 31 days after filing.

☐ Other (specify) _____ **(If less than 31 days after filing, a specific finding under RCW 34.05.380(3) is required and should be stated below)**

Any other findings required by other provisions of law as precondition to adoption or effectiveness of rule?

☐ Yes ☒ No If Yes, explain:

Purpose: Chapter 246-760 WAC – Hearing Screening Standards for School Districts. The purpose of this adopted rule is to update the State Board of Health's (Board's) existing hearing screening standards for school districts. These rules establish requirements for annual preventative hearing and vision screenings, including which students are screened, how often, approved tools, referral criteria, procedures, and staff qualifications. The hearing screening sections of the rule were most recently updated in 2002. Since then, the Board has updated the vision screening sections of the rule, and new hearing screening technology and standards have become available. The adopted updates modernize the Board's hearing screening standards, and will help school districts and screening staff improve hearing screening programs while keeping the rules clear and effective.

Citation of rules affected by this order:

New: WAC 246-760-025

Repealed: N/A

Amended: WAC 246-760-001, WAC 246-760-010, WAC 246-760-020, WAC 246-760-030, WAC 246-760-040, WAC 246-760-050, WAC 246-760-060, WAC 246-760-100

Suspended: N/A

Statutory authority for adoption: RCW 28A.210.020

Other authority: None

PERMANENT RULE (Including Expedited Rule Making)

Adopted under notice filed as **WSR 25-09-146** on **June 4, 2025**.

Describe any changes other than editing from proposed to adopted version: Several minor, non-substantial editorial and clarifying edits were adopted at the June 4th rules hearing, including:

- Revising terminology for consistency and accuracy across rule sections.
- Refining language to reflect the practical realities of school screening environments.
- Clarifying referral procedures to give schools flexibility in how they refer students for further evaluation.

If a preliminary cost-benefit analysis was prepared under RCW 34.05.328, a final cost-benefit analysis is available by contacting:

Name: Molly Dinardo
Address: P.O. Box 47990, Olympia, WA, 98504-7990
Phone: (360) 236-4110
Fax: N/A
TTY: 711
Email: schoolauditoryscreening@sboh.wa.gov or molly.dinardo@sboh.wa.gov
Web site: https://sboh.wa.gov/rulemaking/agency-rules-and-activity/hearing-screening-standards-washington-schools
Other:

Note: If any category is left blank, it will be calculated as zero. No descriptive text.

Count by whole WAC sections only, from the WAC number through the history note. A section may be counted in more than one category.

The number of sections adopted in order to comply with:

Federal statute:	New	0	Amended	0	Repealed	0
Federal rules or standards:	New	0	Amended	0	Repealed	0
Recently enacted state statutes:	New	0	Amended	0	Repealed	0

The number of sections adopted at the request of a nongovernmental entity:

	New	0	Amended	0	Repealed	0

The number of sections adopted on the agency's own initiative:

	New	1	Amended	8	Repealed	0

The number of sections adopted in order to clarify, streamline, or reform agency procedures:

	New	0	Amended	0	Repealed	0

The number of sections adopted using:

Negotiated rule making:	New	0	Amended	0	Repealed	0
Pilot rule making:	New	0	Amended	0	Repealed	0
Other alternative rule making:	New	1	Amended	8	Repealed	0

Date Adopted: 06/04/2025	**Signature:**
Name: Michelle A. Davis	
Title: Executive Director	

Chapter 246-760 WAC
((~~AUDITORY~~)) HEARING AND ((~~VISUAL~~)) VISION SCREENING STANDARDS—SCHOOL DISTRICTS

AMENDATORY SECTION (Amending WSR 17-03-009, filed 1/4/17, effective 7/1/17)

WAC 246-760-001 Purpose and application of ((~~auditory and visual~~)) hearing and vision screening standards for school districts. Each board of school directors in the state shall provide for and require hearing and vision screening of ((~~the auditory and visual acuity of~~)) children attending schools in their districts to determine if any child demonstrates ((~~auditory or visual problems~~)) reduced hearing or vision that may negatively impact their learning. Each board of school directors shall establish procedures to implement these rules.

AMENDATORY SECTION (Amending WSR 17-03-009, filed 1/4/17, effective 7/1/17)

WAC 246-760-010 Definitions, abbreviations, and acronyms. The definitions, abbreviations, and acronyms in this section apply throughout this chapter unless the context clearly requires otherwise.

(1) "AAPOS" or "American Association for Pediatric Ophthalmology and Strabismus" means the national organization that advances the quality of children's eye care, supports the training of pediatric ophthalmologists, supports research activities in pediatric ophthalmology, and advances the care of adults with strabismus.

(2) "ASA/ANSI" or "Acoustical Society of America/American National Standards Institute" means the national organization responsible for publishing standards and technical reports that standardize acoustical terminology and measurements, as well as for developing consensus-driven industry standards.

(3) "Audiometer" means an instrument used to measure hearing acuity. It is commonly used in hearing tests, typically by presenting pure tones, speech signals, or other auditory stimuli to assess changes in a person's hearing ability.

(4) "Audiological evaluation" means a comprehensive diagnostic exam used to determine the type, degree, and configuration of reduction in hearing. This evaluation is performed by a licensed professional or specialist to diagnose and characterize hearing reductions and create an individualized treatment plan to address hearing needs.

(5) "Auditory acuity" or "hearing acuity," refers to how sharp or sensitive someone's hearing is. It can mean the ability to hear faint sounds, distinguish between different sounds (like pitch or loudness), and identify the direction from which a sound is coming from.

(6) "Calibrate" means to adjust and/or verify the accuracy of screening equipment to ensure it meets established standards. This process involves checking and fine-tuning the equipment to ensure it

provides reliable and consistent results in assessing auditory or visual acuity.

(7) "Crowding bars" means four individual lines surrounding a single optotype.

((~~(3)~~)) (8) "Crowding box" or "surround box" means crowding bars on all four sides extended to form a crowding rectangle surrounding a single line of optotypes.

((~~(4)~~)) (9) "dB" or "decibel" means a unit of measurement used to express the relative intensity of sound. It is commonly used to quantify sound levels and describe hearing sensitivity.

(10) "Distance vision" means the ability of the eye to see images clearly at a calibrated distance.

((~~(5)~~)) (11) "Frequencies" refer to the different pitches of sounds, from low (deep) to high (sharp). Hearing is screened across a range of frequencies to identify reduced hearing at one or more frequencies.

(12) "Hearing screening" means a nondiagnostic test to identify if the person being screened needs to be referred for an audiological evaluation.

(13) "Hz" or "hertz" is the standard unit of measurement used for measuring frequency or pitch.

(14) "HOTV letters" means a test using the letters H, O, T, and V calibrated of a certain size used to assess visual acuity.

((~~(6)~~)) (15) "Instrument-based vision screening device" means a U.S. Food and Drug Administration approved instrument for vision screening that uses automated technology to provide information about amblyopia and reduced-vision risk factors such as estimates of refractive error and eye misalignment.

((~~(7)~~)) (16) "Lay person" means any individual who is conducting school-based vision screening other than a school nurse, a school principal or his or her designee, a licensed vision care professional, or an individual trained by and conducting vision screening on behalf of a nationally recognized service organization that utilizes a test-retest protocol for vision screening. This includes, but is not limited to, retired nurses, nursing students, parents, and school staff.

((~~(8)~~)) (17) "LEA vision test(s)" means a test used to measure visual acuity using specific symbols or numbers, designed for those who do not know how to read the letters of the alphabet.

((~~(9)~~)) (18) "Licensed vision care professional" means a licensed ophthalmologist or licensed optometrist.

((~~(10)~~)) (19) "Near vision acuity" means the ability of the human eye to see objects with clarity at close range, also termed near point acuity or near acuity.

((~~(11)~~)) (20) "OAEs" or "otoacoustic emission screening technology or devices" refers to a test that measures the function of the inner ear (cochlea). This technology is commonly used for screening infants and other special populations, particularly when behavioral hearing tests, such as pure tone audiometry, are not appropriate.

(21) "Optotype" means figures, numbers or letters of different sizes used in testing visual acuity.

((~~(12)~~)) (22) "Principal's designee" means a public health nurse, special educator, teacher or administrator designated by the school principal and responsible for supervision, training, reporting and referral of vision screening in instances where the school nurse or school principal is not filling this role.

((~~(13)~~)) (23) "Probe tip" means the part of an OAE screening device inserted into the ear canal to deliver sound and detect inner ear

responses. It must fit snugly and comfortably for accurate screening results.

(24) "School nurse" means a registered nurse acting as the health professional in a school whose specialized practice and attendant tasks and activities advance student health, well-being and achievement; and conforms to Washington state educational and nursing laws according to chapters 18.79 RCW and 246-840 WAC, and WAC 181-79A-223.

((~~(14)~~)) (25) "Sloan letters" means a test using ((~~ten~~)) 10 specially formed letters which include C, D, H, K, N, O, R, S, V and Z to assess visual acuity.

((~~(15)~~)) (26) "Test-retest protocol" means a method of screening where a screener conducts two or more screenings for any student who meets the referral criteria in order to ensure the reliability of the initial screening.

((~~(16)~~)) (27) "Tonal stimuli" refer to sounds with a clear pitch or tone, like a musical note or a beep. These sounds are used in hearing tests to check how well someone can hear.

(28) "Visual acuity" refers to the ability of the visual system to discern fine distinctions in the environment as measured with printed or projected visual stimuli.

AMENDATORY SECTION (Amending WSR 17-03-009, filed 1/4/17, effective 7/1/17)

WAC 246-760-020 ((~~Frequency~~)) Screening requirements for schools ((~~to screen children~~)). ((~~(1) A school shall conduct auditory and distance vision and near vision acuity screening of children:~~

~~(a) In kindergarten and grades one, two, three, five, and seven; and~~

~~(b) Showing symptoms of possible loss in auditory or visual acuity and who are referred to the district by parents, guardians, school staff, or student self-report.~~

~~(2) If resources are available, a school may:~~

~~(a) Expand vision screening to any other grade;~~

~~(b) Conduct other optional vision screenings at any grade using evidence-based screening tools and techniques; or~~

~~(c) Expand vision screening to other grades and conduct optional vision screenings as outlined in (a) and (b) of this subsection.~~

~~(3) If resources permit, schools shall annually conduct auditory screening for children at other grade levels.~~)) (1) Schools shall conduct annual screening for hearing and vision (both near and distance) for students:

(a) In kindergarten and in grades one, two, three, five, and seven; and

(b) Showing signs of possible reductions in auditory or visual acuity that may negatively impact their learning, or those referred to the district by parents, guardians, school staff, etc.

(2) If resources are available, a school may:

(a) Expand screenings to other grades;

(b) Conduct additional optional vision screenings at any grade using evidence-based screening tools and techniques; or

(c) Both expand screenings to other grades and conduct optional vision screenings as outlined in (a) and (b) of this subsection.

((~~AUDITORY ACUITY~~)) HEARING SCREENING STANDARDS

NEW SECTION

WAC 246-760-025 Hearing screening. (1) A school shall conduct all hearing screenings using tools and procedures that are linguistically, developmentally, and age-appropriate, and shall use screening tools identified in WAC 246-760-030.

(2) A school shall conduct hearing screening according to the tool's instructions and screening protocol.

(3) A school is not required to screen a student who has already had a comprehensive audiological evaluation by a licensed professional within the last 12 months. Schools need a report or form signed by a licensed professional to waive the screening, indicating that an examination has been administered. A school must place this report or form in the student's health record.

(4) A school is not required to screen a student reported by the school district as having reduced hearing levels, as required under RCW 72.40.060.

(5) Exempt students may request to participate in hearing screenings to promote inclusion and prevent stigmatization.

AMENDATORY SECTION (Amending WSR 02-20-079, filed 9/30/02, effective 10/31/02)

WAC 246-760-030 ((~~What are the auditory acuity screening standards for screening equipment and procedures?~~)) Required and alternative hearing screening tools. (1) Schools shall use ((~~auditory~~)) hearing screening equipment ((~~providing~~)) that delivers tonal stimuli at frequencies ((~~at one thousand, two thousand, and four thousand herz~~)) of 1,000, 2,000, and 4,000 hertz (Hz) at ((~~hearing~~)) a sound level((~~s~~)) of ((~~twenty~~)) 20 decibels (dB), ((~~as~~)) measured at the earphones, ((~~in reference to~~)) consistent with Acoustical Society of America (ASA)/American National Standards Institute (ANSI) ((~~1996~~)) S3.6-2018 (R 2023) standards.

(2) Qualified persons will check the calibration of frequencies and intensity at the earphones at least ((~~every twelve months, at the earphones,~~)) once a year using equipment designed for audiometer calibration.

(3) Otoacoustic emission (OAE) screening devices may be used to screen students who cannot participate in pure tone hearing screening including, but not limited to:

(a) Students with special health care needs;

(b) Students with developmental delays or disabilities;

(c) Students who speak a language other than English;

(d) Students who are not old enough or have difficulty understanding the screener's instructions.

(4) OAE screening devices shall not replace screening using pure tone hearing screening equipment except as described in subsection (3)(a) through (d) of this section.

(5) If schools use OAE devices for students who cannot participate in pure tone hearing screening, they shall use calibrated equipment that delivers appropriate stimuli and pass/refer criteria.

(a) The tonal stimuli used during the test must be:

(i) 65/55 dB for distortion product otoacoustic emissions (DPOAEs); or

(ii) 80 dB for transient evoked otoacoustic emissions (TEOAEs).

(b) For a pass result, the screening device must show a response at least three dB louder than the background noise at a minimum of three different frequencies, ranging from 2,000 Hz to 8,000 Hz.

AMENDATORY SECTION (Amending WSR 02-20-079, filed 9/30/02, effective 10/31/02)

WAC 246-760-040 ((~~What are the procedures for auditory acuity screening?~~)) Hearing screening procedures. (1) Schools shall screen all ((~~children~~)) students referenced in WAC 246-760-020 ((~~on an individual basis at one thousand, two thousand, and four thousand~~)) using hearing screening equipment that delivers tonal stimuli at 1,000, 2,000, and 4,000 Hz.

(2) The screener shall:

(a) Conduct screenings in an environment free of extraneous noise, to the extent possible in a school setting;

(b) Position the student so they cannot see the front of the hearing screening equipment or are not facing it;

(c) Present each ((~~of the tonal stimuli~~)) tone at a hearing level of ((~~twenty~~)) 20 dB ((~~based on the~~)), following ASA/ANSI ((~~1996~~)) 2023 standards;

((~~(b) Conduct screenings in an environment free of extraneous noise;~~

~~(c)~~)) (d) Reinstruct the student or reposition the earphones if they appear confused or do not respond to the tonal stimuli;

(e) If at all possible, complete screening within the first semester of each school year;

((~~(d)~~)) (f) Place the results of screenings, any referrals, and referral results in each student's health and/or school record; and

((~~(e)~~)) (g) Forward the results to the student's new school if the student transfers.

(3) If a student cannot participate in pure tone hearing screening, an OAE device may be used. For screeners using OAE devices, they shall:

(a) Examine the student's ear to select an appropriately sized probe tip that fits comfortably and securely in the ear canal;

(b) Insert the probe into the student's ear canal and begin the screening. Make sure the equipment shows that the probe is securely in place and that the student is calm and still. For the best results, the screener should help the student stay quiet and keep the probe steady during the test;

(c) Continue measuring the OAE response until the equipment shows either a "PASS" or "REFER" result.

AMENDATORY SECTION (Amending WSR 02-20-079, filed 9/30/02, effective 10/31/02)

WAC 246-760-050 ((~~What are the auditory acuity~~)) Hearing screening referral procedures((~~?~~)). (1) If a ((~~child~~)) student does not respond to one or more frequencies in either ear during a hearing screening or gets a "refer" result from an OAE:

(a) The school must rescreen the ((~~child~~)) student within six weeks, allowing a minimum of one to two weeks between screenings when possible; and

(b) The school must notify ((~~their~~)) the student's teachers ((~~of~~)) about the need for preferential ((~~positioning~~)) seating in class ((~~because of~~)) due to the possibility of decreased hearing; and

(c) If the student's results indicate that further assessment or follow-up is needed, the school shall notify the parents or legal guardian ((~~of the need for audiological evaluation if the student fails the second screening~~)) that a comprehensive evaluation may be necessary. This evaluation may be preceded by a medical assessment to rule out other factors and to access audiology services as needed.

(d) If a school district utilizes OAE devices as part of its hearing screening procedures, the school shall identify and document the specific type of screening device used.

(2) The school((~~s~~)) shall notify parents or legal guardians ((~~of the need for medical~~)) if a comprehensive evaluation ((~~if~~)) is needed when:

(a) ((~~Indicated by audiological evaluation~~)) The results of a hearing screening suggest it; or

(b) ((~~Audiological evaluation is not available~~)) A school or school district does not have access to an audiologist on staff.

AMENDATORY SECTION (Amending WSR 02-20-079, filed 9/30/02, effective 10/31/02)

WAC 246-760-060 ((~~What are the auditory acuity~~)) Hearing screening ((~~qualifications for~~)) personnel((~~?~~)) qualifications. Each school district shall designate a district audiologist, school nurse, speech language pathologist, health assistant or ((~~district~~)) other staff member ((~~having~~)) to be responsible for the hearing screening program. This person must:

(1) ((~~Responsibility for administering the auditory~~)) Oversee the hearing screening program; and

(2) Have the training and experience to:

(a) ((~~Develop~~)) Create an administrative plan for conducting ((~~auditory~~)) annual hearing screenings ((~~in cooperation with the~~)) and work with appropriate school ((~~personnel~~)) staff to ensure the program is carried out efficiently and effectively;

(b) Obtain and maintain the necessary ((~~instrumentation for carrying out the screening program, and~~)) screening equipment ensuring ((~~the equipment~~)) it is calibrated correctly and in ((~~proper~~)) good working order ((~~and calibration~~)); and

(c) ((~~Secure~~)) Recruit appropriate personnel for carrying out the screening program, if assistance is necessary, and ((~~for assuring~~)) assure these personnel are sufficiently trained to:

(i) Understand the purpose((~~s~~)) and regulations ((~~involved in~~)) of the ((~~auditory~~)) hearing screening program((~~s~~)); and

(ii) ((~~Utilize~~)) Use the screening equipment ((~~to ensure maximum accuracy~~)) properly to get accurate results;

(d) Ensure screening records are ((~~made~~)) created and distributed as appropriate; and

(e) Disseminate information to other school ((~~personnel familiarizing~~)) staff to familiarize them with aspects of a ((~~child's~~)) student's behavior ((~~indicating~~)) that may indicate the need for referral for ((~~auditory~~)) hearing screening.

The person designated as responsible for the hearing screening program must be sufficiently trained to meet the provisions in (c) of this subsection if they are involved in carrying out the screening program.

((~~VISUAL ACUITY~~)) VISION SCREENING STANDARDS

AMENDATORY SECTION (Amending WSR 17-03-009, filed 1/4/17, effective 7/1/17)

WAC 246-760-100 Qualifications for ((~~the visual acuity~~)) vision screening personnel. (1) Persons performing visual screening may include, but are not limited to, school nurses, school principals, other school personnel, or lay persons who have completed training in vision screening; and ophthalmologists, optometrists, or opticians who donate their professional services to schools or school districts. If an ophthalmologist, optometrist, or optician who donates his or her services identifies a visual problem that may impact a student's learning, the vision professional shall notify the school nurse, or the school principal or his or her designee of the results of the screening in writing but may not contact the student's parents or guardians directly per RCW 28A.210.020.

(2) Screening must be performed in a manner consistent with this chapter and RCW 28A.210.020. Any person conducting vision screening must be competent to administer screening procedures as a function of their professional training and background or special training and demonstrated competence under supervision by the school nurse, or the school principal or his or her designee.

(3) A lay person shall demonstrate his or her competence at administering the screening tools including controlling for lighting or distractions that could affect the screening results.

(4) Supervision, training, reporting and referral of vision screening shall be the responsibility of the school nurse, or the school principal or his or her designee. The principal or his or her designee must demonstrate his or her competence in vision screening through supervised training by a competent school or public health nurse or licensed vision care professional, have supervisory ability and experience, and have the ability to work well with school staff

and lay persons. Ideally, the person should demonstrate the ability to teach vision screening techniques and operations to others.

(5) Students in grades kindergarten through ((~~twelve~~)) 12 may not assist with or conduct vision screening of other students in their school district, unless students are supervised and conducting screening within the scope of an advanced vocational health-related curriculum such as nursing.

RULE-MAKING ORDER
PERMANENT RULE ONLY

CR-103P (December 2017)
(Implements RCW 34.05.360)

CODE REVISER USE ONLY

OFFICE OF THE CODE REVISER
STATE OF WASHINGTON
FILED

DATE: June 18, 2025
TIME: 9:59 AM

WSR 25-13-109

Agency: State Board of Health

Effective date of rule:

Permanent Rules

☐ 31 days after filing.

☒ Other (specify) September 1, 2025 **(If less than 31 days after filing, a specific finding under RCW 34.05.380(3) is required and should be stated below)**

Any other findings required by other provisions of law as precondition to adoption or effectiveness of rule?

☐ Yes ☒ No If Yes, explain:

Purpose: Primary and Secondary School Environmental Health and Safety Regulations. The State Board of Health (Board) is repealing chapter 246-366A and WAC 246-366-160 and amending WAC 246-366-005 to remove references to chapter 246-366A. By amending and repealing these sections of rule and chapter, the Board will prevent the implementation of rules that are out-of-date and obsolete. Current requirements in chapter 246-366 WAC will remain in effect.

Citation of rules affected by this order:

New: None

Repealed: WAC 246-366-160, 246-366A-001, 246-366A-003, 246-366A-005, 246-366A-010, 246-366A-015, 246-366A-020, 246-366A-030, 246-366A-040, 246-366A-050, 246-366A-060, 246-366A-065, 246-366A-070, 246-366A-080, 246-366A-090, 246-366A-095, 246-366A-100, 246-366A-105, 246-366A-110, 246-366A-115, 246-366A-120, 246-366A-125, 246-366A-130, 246-366A-135, 246-366A-140, 246-366A-150, 246-366A-155, 246-366A-160, 246-366A-165, 246-366A-170, 246-366A-175, 246-366A-180, 246-366A-190, and 246-366A-200

Amended: WAC 246-366-005

Suspended: None

Statutory authority for adoption: RCW 43.20.050 (2)(d)

Other authority: N/A

PERMANENT RULE (Including Expedited Rule Making)

Adopted under notice filed as WSR 25-09-121 on April 21,2025 .

Describe any changes other than editing from proposed to adopted version:

The Board made no changes from the proposed version to the adopted version.

If a preliminary cost-benefit analysis was prepared under RCW 34.05.328, a final cost-benefit analysis is available by contacting:

Name:
Address:
Phone:
Fax:
TTY:
Email:
Web site:
Other:

Note: If any category is left blank, it will be calculated as zero. No descriptive text.

Count by whole WAC sections only, from the WAC number through the history note. A section may be counted in more than one category.

The number of sections adopted in order to comply with:

Federal statute:	New	0	Amended	0	Repealed	0
Federal rules or standards:	New	0	Amended	0	Repealed	0
Recently enacted state statutes:	New	0	Amended	0	Repealed	0

The number of sections adopted at the request of a nongovernmental entity:

	New	0	Amended	0	Repealed	0

The number of sections adopted on the agency's own initiative:

	New	0	Amended	1	Repealed	34

The number of sections adopted in order to clarify, streamline, or reform agency procedures:

	New	0	Amended	0	Repealed	0

The number of sections adopted using:

Negotiated rule making:	New	0	Amended	0	Repealed	0
Pilot rule making:	New	0	Amended	0	Repealed	0
Other alternative rule making:	New	0	Amended	1	Repealed	34

Date Adopted: June 5, 2025 **Name:** Michelle Davis **Title:** Executive Director	**Signature:** 

AMENDATORY SECTION (Amending WSR 10-01-174, 10-12-018, 11-10-080, 13-09-040, 15-09-070, 17-14-055, 19-14-107, 21-14-056, 22-14-021, 23-16-005, and 24-14-089, filed 12/22/09, 5/21/10, 5/3/11, 4/11/13, 4/15/15, 6/28/17, 7/2/19, 7/1/21, 6/24/22, 7/19/23, and 6/28/24, effective 9/1/25.)

WAC 246-366-005 Purpose. The purpose of this chapter is to maintain minimum environmental health and safety standards for school facilities until legislative action allows for full or partial implementation of ((~~chapter 246-366A WAC. To the extent the legislature funds or otherwise allows for its implementation, chapter 246-366A WAC is~~)) new standards, intended to replace or supersede this chapter.

REPEALER

The following sections of the Washington Administrative Code are repealed:

WAC 246-366-160 Severability.

REPEALER

The following chapter of the Washington Administrative Code is repealed:

WAC 246-366A-001	Introduction and purpose.
WAC 246-366A-003	Implementation.
WAC 246-366A-005	Applicability.
WAC 246-366A-010	Definitions.
WAC 246-366A-015	Guidance.
WAC 246-366A-020	Responsibilities—General.
WAC 246-366A-030	Site assessment, review, and approval.
WAC 246-366A-040	Construction project review.
WAC 246-366A-050	Preoccupancy inspection of construction projects.
WAC 246-366A-060	General construction requirements.
WAC 246-366A-065	General operation and maintenance requirements.
WAC 246-366A-070	Moisture control, mold prevention, and remediation.
WAC 246-366A-080	Safety—Animals in school facilities.
WAC 246-366A-090	Heating and ventilation—Construction requirements.
WAC 246-366A-095	Heating and ventilation—Operation and maintenance requirements.
WAC 246-366A-100	Noise control—Construction requirements.
WAC 246-366A-105	Noise control—Operation and maintenance requirements.
WAC 246-366A-110	Lighting—Construction requirements.
WAC 246-366A-115	Lighting—Operation and maintenance requirements.
WAC 246-366A-120	Restrooms and showers—Construction requirements.
WAC 246-366A-125	Restrooms and showers—Operation and maintenance requirements.
WAC 246-366A-130	Water quality monitoring—Lead.
WAC 246-366A-135	Water quality monitoring—Copper.
WAC 246-366A-140	Water quality monitoring—Other drinking water contaminants.
WAC 246-366A-150	Playgrounds—Construction and installation requirements.
WAC 246-366A-155	Playgrounds—Operation and maintenance requirements.
WAC 246-366A-160	Laboratories and shops—Construction requirements.

WAC 246-366A-165	Laboratories and shops——Operation and maintenance requirements.
WAC 246-366A-170	Variances.
WAC 246-366A-175	Temporary emergency waivers for disaster situations.
WAC 246-366A-180	Appeals.
WAC 246-366A-190	Complaints.
WAC 246-366A-200	Severability.

WASHINGTON STATE BOARD OF HEALTH

Board of Health Legislative Report
WAC 246-370 School Environmental Health and Safety Rule



The School Rule Legislative Report details the technical advisory committee's comprehensive review of the state's outdated school environmental health and safety rule. It highlights key issues identified during the development of a new set of minimum public health and safety standards. It candidly discusses challenges that emerged, including some outside the direct scope of the Board's authority and the proposed rule. It recommends a phased approach to implementation that balances the greatest health and safety benefits with the cost of implementation.

WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Legislative Report
Branch-Chain Ketoacid Dehydrogenase Kinase (BCKDK) Deficiency



Newborn Screening Legislative Report
Branch-Chain Ketoacid Dehydrogenase Kinase (BCKDK) Deficiency

June 2025

WASHINGTON STATE BOARD OF HEALTH

The Board, with the support of the Department of Health, evaluates potential new conditions through a defined process and criteria involving evidence, ethics, equity, and cost-effectiveness. As directed by SB 6234 (Chapter 105, Laws of 2024), the Board has submitted its final report on Branched-Chain Ketoacid Dehydrogenase Kinase (BCKDK). The report includes the Board's findings and recommendation **not** to add BCKDK deficiency to the state's mandatory newborn screening panel.

WASHINGTON STATE BOARD OF HEALTH

Hearing Screening CR-103 Announcement



On June 4, 2025, the Board approved updates to its Hearing Screening Standards for School Districts. To help improve access to this information, staff created a short video explaining the changes. The video was shared through the Board's distribution lists and with interested parties via GovDelivery.

WASHINGTON STATE BOARD OF HEALTH

LOCAL BOARDS OF HEALTH - TRIBAL MEMBERSHIP (ESHB 1946)

Engrossed Substitute House Bill (ESHB) 1946 passed during the 2025 legislative session. This law requires the State Board of Health (Board) to clarify its local board of health (LBOH) composition rules and timelines for including Tribal representation on LBOHs (**Chapter 246-90 WAC**). The law also requires LBOHs to maintain a balance between elected and non-elected members. The law reinforces Tribal Sovereignty and self-determination by shifting the appointment of Tribal members from the American Indian Health Commission (AIHC) to the Tribes and Urban Indian organizations within a health jurisdiction.

This law is effective July 27, 2025. LBOHs must follow this law when adding non-elected members, including Tribal representatives to their boards. After July 27, 2025, LBOHs must add Tribal members to their local boards of health within 60 days of receiving an appointment by a Tribe or Urban Indian health organization. The law requires the Board to establish timelines for modifying local board of health membership in chapter 246-90 WAC by July 27, 2026.

This law:

- Gives the Board 12 months to complete the update of the local board of health (LBOH) composition rules to reflect the changes required by ESHB 1946.
- Clarifies the requirements for Tribal representation on LBOHs.
- Requires the Board to adopt rules to establish timelines for adjusting LBOH membership to keep a balanced mix of elected and non-elected members when Tribal representatives are added.
- Expands representation to ensure Tribes and Urban Indian organizations recognized by the Indian Health Service and registered as a 501(c)(3) within a jurisdiction, choose their representative for LBOHs or health district boards.
- Shifts the appointment authority from the American Indian Health Commission (AIHC) to the Tribes and Urban Indian organizations, respecting Tribal sovereignty and self-determination.
- Requires that the LBOH notify the AIHC when a Tribe or Urban Indian organization appoints a Tribal representative. Previously, the law required the AIHC to select Tribal representatives for LBOHs, where applicable.

STATE OF WASHINGTON

DEPARTMENT OF HEALTH

PO Box 47890 • Olympia, Washington 98504-7890
Tel: 360-236-4030 ● 711 Washington State Relay

August 11, 2025

K.D. Chapman-See
Director
Office of Financial Management
Post Office Box 43113
Olympia, Washington 98504-3113

Re: Governmental Public Health System Concurrence on FPHS Investments of **$150,115,000** for SFY26 – Transfer Request to Department of Health (DOH) for Foundational Public Health Services (FPHS)

Dear K.D.:

The Washington State Department of Health (DOH), in partnership with the state's governmental public health system – the Washington State Board of Health, local health jurisdictions, and sovereign tribal nations and tribal organizations, request appropriation from the Office of Financial Management to DOH in the sum of **$129,091,000 in general fund-state spending authority and $21,024,000 of Dedicated FPHS Account authority for SFY26.** This amount shall be used to fund foundational public health service (FPHS) activities that support the governmental public health system.

- The exhibits below illustrate how the funds will be allocated. DOH will contract out the funds using the standard contracting process.

- The requirements allowing this transfer as set forth in RCW 43.70.515 have been met.

The governmental public health system is requesting funds for SFY26 instead of the entire 2025-2027 biennium due to forecasted revenue projections that will require additional concurrence discussions of the FPHS Steering Committee for SFY27 investments.

Certified Agreement

DOH completed a formal consultation with federally recognized sovereign tribal nations on June 11, 2025. The tribal consultation took place in accordance with Chapter 43.376 RCW, the Washington State Centennial Accord of 1989, and the DOH Consultation and Collaboration Procedure, which recognizes the government-to-government relationship with sovereign tribal nations in the development of policies, agreements, and program implementation that directly affect tribes and tribal people.

The results of this consultation were shared with the FPHS Steering Committee at their June 18, 2025, meeting.

The entities required for certification – DOH, State Board of Health, sovereign tribal nations, and a state association representing local health jurisdictions (Washington State Association of Local Public Health Officials)– are jointly certifying this agreement by the signatures to this letter.

Thank you for your continued partnership to strengthen our state's governmental public health system.

Respectfully,

Dennis E. Worsham
Secretary of Health
Department of Health

Michelle Davis, MPA
Executive Director
State Board of Health

Carla Prock, RN BSN
President
Washington State Association
of Local Public Health Officials

Steve Kutz, BSN, MPH
Chairperson
American Indian Health
Commission of Washington State

cc: Jaime Bodden, Managing Director, WA State Association of Local Public Health Officials
Kelly Cooper, Director, Office of Policy & Legislative Relations, DOH
Amy Ferris, Chief Financial Officer, Office of Financial Services, DOH
Amy Hatfield, Budget Advisor, Budget Office, OFM
Cynthia Hollimon, Senior Budget Advisor, Budget Office, OFM
Jeff Ketchel, Director, Foundational Public Health Services, DOH
Vicki Lowe, Executive Director, American Indian Health Commission
Kristin Peterson, Chief, Office of Policy, Planning & Evaluation, DOH
Caitlin Safford, Senior Policy Advisor, Executive Policy Office, GOV
Steve Smothers, Special Projects, Foundational Public Health Services, DOH
Jessica Todorovich, Chief of Staff, Office of the Secretary, DOH
Derek Young, Executive Director, WA State Association of Counties

SFY 2026 | Exhibit 1 – FPHS Fiscal Analysis Overview by Sector

AGENCY	TOTAL ALLOCATION
Department of Health (DOH) Total	$31,148,000
WSALPHO and Local Health Jurisdiction (LHJ) Total	$110,702,000
Tribes and Tribal Organizations Total	$7,080,000
State Board of Health (SBOH) Total	$1,185,000
TOTAL ANNUAL ALLOCATION	**$150,115,000**

SFY 2026 | Exhibit 2 – FPHS Area by Agency

Sector	FPHS Area	SFY26 Allocation
DOH	Assessment (Surveillance & Epidemiology)	$6,020,677
	CCC = Foundational Capabilities (Communication, Policy, Partnership, & Business Competencies)	$1,412,000
	CD = Communicable Disease	$17,409,323
	EH = Environmental Health	$4,801,000
	EP = Emergency Preparedness	$1,019,500
	LC - Lifecourse	$485,500
DOH Total		**$31,148,000**
Local Health Jurisdictions (35 total)	Assessment (Surveillance & Epidemiology)	$4,664,000
	CCC = Foundational Capabilities (Communication, Policy, Partnership, & Business Competencies)	$49,015,000
	CD = Communicable Disease	$18,337,000
	EH = Environmental Health	$8,092,000
	EP = Emergency Preparedness	$6,023,000
	LC = Lifecourse	$23,496,000
LHJ Total		**$109,627,000**
SBOH	CCC = Foundational Capabilities (Communication, Policy, Partnership, & Business Competencies)	$1,185,000
SBOH Total		**$1,185,000**
Tribes	Funds to deliver culturally appropriate foundational public health services	$5,800,000
Tribes Total		**$5,800,000**

Tribal Org	Funds to deliver culturally appropriate foundational public health services	$1,280,000
Tribal Org Total		**$1,280,000**
WSALPHO	CCC = Foundational Capabilities (Communication, Policy, Partnership, & Business Competencies)	$350,000
	CD = Communicable Disease	$300,000
	LC = Lifecourse	$425,000
WSALPHO Total		**$1,075,000**
Grand Total		**$150,115,000**

WASHINGTON STATE BOARD OF HEALTH

Date: August 20, 2025

To: Washington State Board of Health Members

From: Michelle Davis, Executive Director

Subject: Department of Health Fluoride Science Review Update

Background and Summary:

The Board has the authority under RCW 43.20.050 to adopt rules for Group A public water systems as defined in RCW 70A.125.010. Chapter 246-290 WAC establishes the standards for these water systems related to their design, construction, sampling, management, maintenance, and operation practices. The purpose of these rules is to define basic regulatory requirements and to protect the health of consumers using public drinking water supplies. They include maximum contaminant levels for fluoride and an optimal level of fluoridation for public water systems that choose to fluoridate.

Decisions about whether to fluoridate public water systems are generally made at the local level. RCW 70.05.060(3) authorizes local boards of health to enact rules necessary to preserve and improve public health, including whether to require fluoridation of public water systems within their jurisdiction (AGO 2008 No.5). In addition, RCW 57.08.012 authorizes fluoridation of a water district's water by a majority vote of water district commissioners.

In 2024, the Board received multiple rulemaking petitions requesting changes to WAC 246-290-220, Drinking Water Materials and Additives, under the Group A Public Water Supplies rules. The petitioner proposed adding new language to the rule related to fluoride exposure during pregnancy and early childhood. The petitioner also recommended against adding fluoride or other chemicals to drinking water to treat or prevent disease, citing findings from the National Toxicology Program (NTP) showing an association between high levels of fluoride exposure and lowered IQ in children.

In August 2024, the National Toxicology Program published the Monograph on the *State of the Science Concerning Fluoride Exposure and Neurodevelopment and Cognition: A Systematic Review* (Monograph). The Monograph found, with moderate confidence, that higher levels of fluoride exposure, such as drinking water containing more than 1.5 milligrams of fluoride per liter, are associated with lower IQ in children.

In response to the emerging research and petition requests, the Department of Health (Department) convened a Fluoride Science Review Panel, consisting of experts from across the public health system, to review the emerging science and evidence in the petitions and any additional fluoride-related research.

(continued on the next page)

Department representatives Dr. Tao Kwan-Gett, State Health Officer, and Assistant Secretary for Environmental Public Health, Lauren Jenks, will brief the Board on the findings and recommendations of the panel. Following their presentation, Board Staff will present information related to Board authority and other considerations.

Recommended Board Actions:

The Board may wish to consider and amend, if necessary, the following motions:

Direct Staff to remove the Recommended Strategies to Improve the Oral Health of Washington Residents (2015) document and accompanying webpage from the Board's website, and work with the Department of Health to review and update the document's content and messaging.

AND

Direct Staff to brief the Board at a future meeting regarding a potential rulemaking to consider adopting a State Advisory Level (SAL) or State Maximum Contaminant Level (MCL) for fluoride.

Staff

Shay Bauman, Policy Advisor

To request this document in an alternate format or a different language, please contact the Washington State Board of Health at 360-236-4110 or by email at wsboh@sboh.wa.gov. TTY users can dial 711.



Washington State Department of HEALTH

FLUORIDE SCIENCE REVIEW

Tao Sheng Kwan-Gett, MD, MPH
Lauren Jenks, MPH, CHES

Oral Health is Essential

- Oral health impacts physical and mental health, school and work attendance, and many aspects of quality of life.
- There are strong associations between dental caries and stroke and all-cause mortality.
- Oral disorder costs make up about 3.8% of US health care spending, with an estimated cost of $93 billion.



Role of Department of Health

Office of Drinking Water

- Provides technical assistance to water systems that decide to optimally fluoridate their water.
- Ensures that naturally occurring fluoride in the water stays below the EPA's maximum contaminant level (MCL).

Office of Healthy and Safe Communities, State Oral Health Program:

- Promotes strategies that protect oral health, including the benefits of fluoride for the prevention and management of dental decay.
- Occasionally provides technical assistance to communities about community water fluoridation.

Prevention and Community Health Division:

- Works to prevent disease and promote a healthy start, healthy choices, and access to services for children and families, including during pregnancy.

Role of the State Board of Health

Washington state law (RCW 57.08.012) allows, but does not require, community water fluoridation.

The State Board of Health sets an optimal level of fluoridation for water systems that choose to provide fluoridated water.

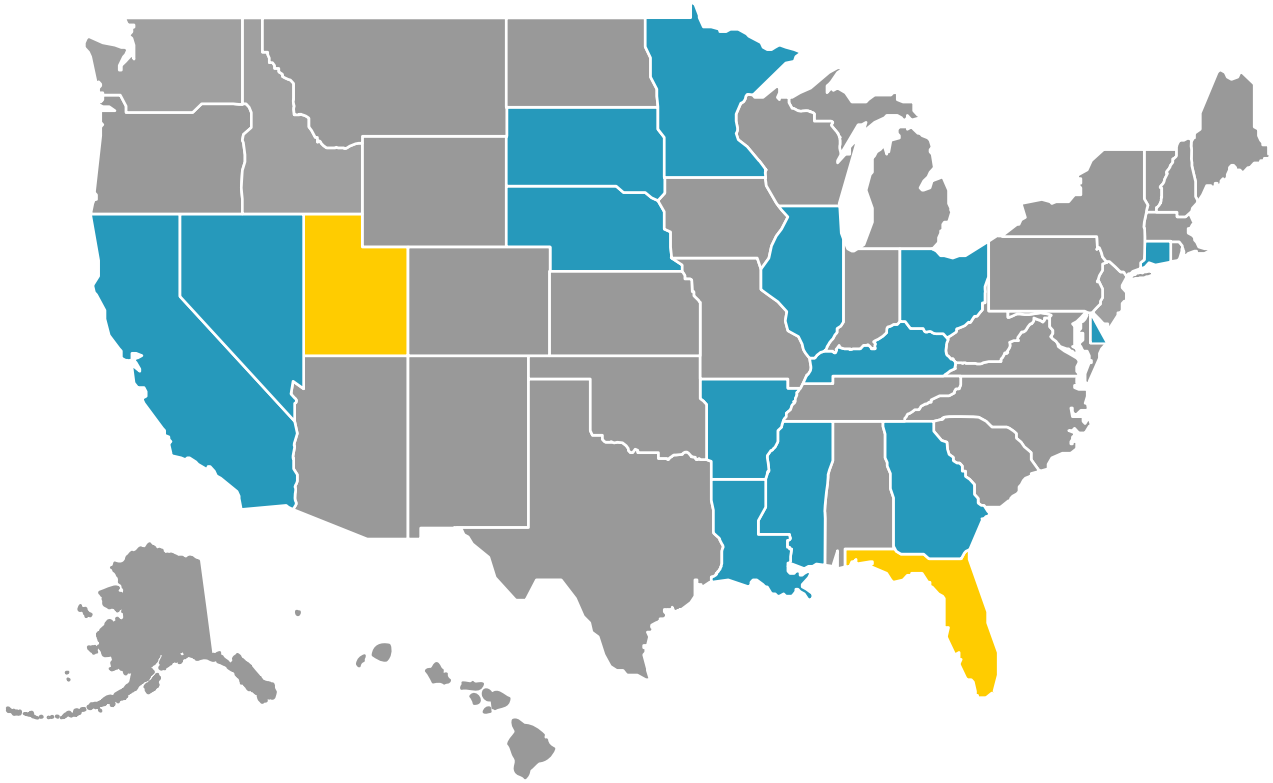
The State Board of Health sets a maximum contaminant level, or a state action level.

SAL Exceedance:	**MCL Exceedance:**
• **Notify DOH** • **Notify water users** • **Continue monitoring** • **Investigate cause of contamination** • **Take action as directed by department**	• **Notify DOH** • **Notify consumers** • **Investigate cause of contamination** • **Take action as directed**

Fluoridation Policy by State

States which require or prohibit fluoridation in some form

(July 2025)



Endorsements

The Centers for Disease Control and Prevention

American Medical Association

American Academy of Pediatrics

American Dental Association

American Academy of Pediatric Dentistry

American Association for Dental, Oral, and Craniofacial Research



Fluoride Science Review Panel

- The panel was charged with listening, learning, and considering all relevant science in their discussions of community water fluoridation.
- Convened January through June 2025
- Panel members represented governmental public health in Washington:
  - Department of Health
  - Local Health Jurisdictions
  - The State Board of Health
  - Tribal Health Organizations

Limitations

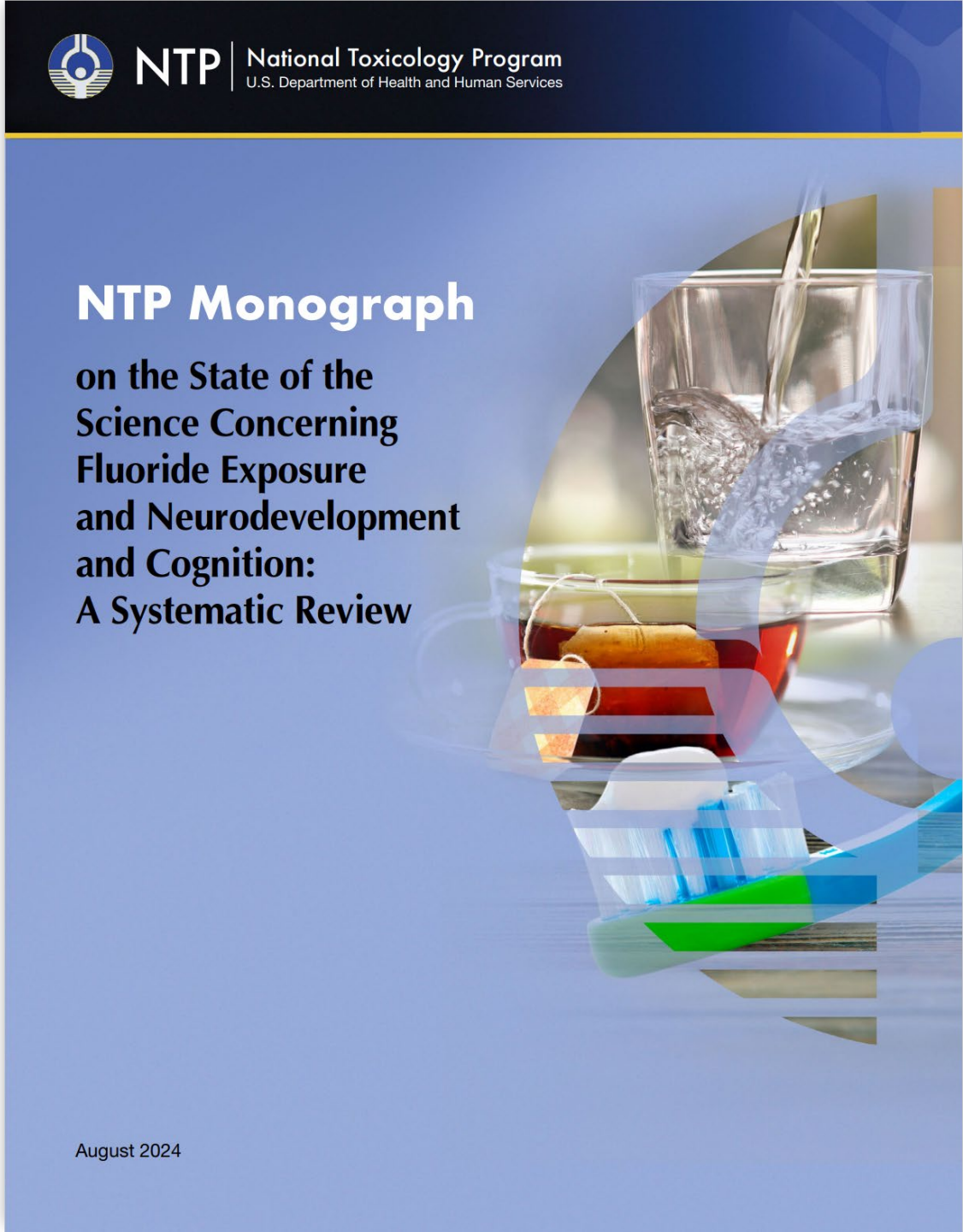
- Did not assess evidence around other strategies for prevention of tooth decay
- Did not assess evidence around healthy brain development or measurement of IQ in children
- Did not assess potentially less risky methods of getting benefits of fluoride
- Did not consider changes to the health care system or dental care system



Information Reviewed

Fluoride, Neurodevelopment, and Cognition: A National Toxicology Program Monograph

- Moderately sure: that higher estimated fluoride exposures are consistently associated with lower IQ in children.
- Unsure: whether low fluoride level of 0.7 mg/L currently recommended for US community water has a negative effect on children's IQ.
- No evidence: that fluoride exposure has adverse effects on adult cognition.





Information Reviewed

2024 federal district court order on fluoride:

Fluoridation of water at 0.7 mg/L poses an unreasonable risk of reduced IQ in children.

- Does not mean that the court found that fluoridated water is definitely harmful. Rather that the court found an unreasonable risk of harm, a standard used by EPA under TSCA.
- The court did not consider the benefits of fluoride in their review.
- A court finding is not a scientific finding. It is an interpretation of the science that exists in reference to current federal law

Information Reviewed

Oral Health Disparities and Access

- Oral health impacts physical and mental health, school and work attendance, and many aspects of quality of life.
- Exposure to fluoride hardens the tooth enamel and is protective against tooth decay in children and adults.
- Community water fluoridation is a long-standing intervention intended to help large portions of the community, regardless of access to dental care or fluoride-containing hygiene products.
- Access to dental care varies by employment and income and location in the state. Many children and adults in the state lack adequate access to dental care.





Information Reviewed

2024 Cochrane Review: *Water fluoridation for the prevention of dental caries*

- Adding fluoride to water may slightly increase the number of children who have no tooth decay in either their baby teeth or permanent teeth. However, these results also included the possibility of little or no difference in tooth decay.
- **Unsure**: whether adding fluoride to water reduced tooth decay in children's permanent teeth.
- **Unsure**: whether there are any effects on tooth decay when fluoride is removed from a water supply.
- **Unsure**: whether fluoride reduces differences in tooth decay between people with higher incomes and people with lower incomes.



Information Reviewed

Case Study: Calgary, Canada

- Fluoride was introduced to drinking water in 1991, removed in 2011, and reintroduced in 2021.
  - Discontinuing community water fluoridation was associated with increased dental treatment under general anesthesia, especially among children 0-5 years old.
  - Odds of untreated dental decay increased more among those without dental insurance from 2009/2010 to 2013/2014, showing an increase in disparity.
  - The authors present multiple possible causes, one of which is the end of community water fluoridation.

Information Reviewed

Case Study: Juneau, Alaska

- Increased dental care costs were correlated with the stopping of community water fluoridation
- After stopping community water fluoridation, there were significant differences in the mean number of Medicaid eligible dental procedures among 0-6, 0-7, and 0-18 year-olds, but not significant in ages 7-13 or 13-18.
- This indicates a significant difference in treatment of primary teeth but not permanent teeth.



Information Reviewed

Economic Analysis

Most studies about fluoride and economic costs focus on:

- Saving on dental care costs.
- Avoiding lost work time.
- How much it costs water systems to add fluoride.
- Two recent papers call for inclusion of the costs of treating fluorosis and/or lost IQ points and demonstrate a reduction in the historical return on investment.

Most studies do not count the cost of treating fluorosis or possible IQ loss.

Even with the decline of benefits from community water fluoridation, literature continues to report it as a cost-effective intervention.



Information Reviewed

Dr. Christine Till presented an overview of the emerging science on fluoride toxicology:

A 1 mg/L increase in fluoride intake was associated with a 3.66 (95% CI,-7.16 to -0.15; p=.04) lower IQ score in boys and girls.

Formula-fed babies are at risk of lower IQ if their formula is made with fluoridated water.

Evidence of fluoride neurotoxicity at urine fluoride levels <1.5 mg/L is relevant to community water fluoridation because pregnant women and children can exceed an equivalent dose of fluoride even when drinking optimally fluoridated water depending on amount of fluoridated water they ingest and their exposure to other sources of fluoride.

Till stated, “Given that fluoride offers little benefit to the fetus and young infant, community-wide administration of systemic fluoride may pose an unfavorable risk-benefit ratio for the pregnant woman, fetus, and infant.”

Fluoride is not a chemical; it’s a natural substance.

Community water fluoridation is endorsed by CDC, AMA, AAP, ADA, AAPD.

Community water fluoridation is a population-based intervention that helps everyone.

There is no association between community water fluoridation and IQ.

As feds cut Medicaid, community water fluoridation will be more important for high-risk communities.

Epidemiologic evidence informs our understanding that fluoride is beneficial throughout the lifespan.

Community Input

Dentists, advocates, researchers, and concerned members of the public shared their views with the panel.

> The State Board of Health must ensure safe drinking water.

> Fluoride is not good for everyone. Some people experience unique toxic effects.

> Putting fluoride in water is not effective at preventing cavities, but it presents an unacceptable neurodevelopmental risk.

> We should follow the precautionary principle and not expose people to a chemical we cannot prove is safe for everyone.

> People should be able to opt out of fluoride if they don't want it.

> There is no reason for babies under 6 months of age to get any fluoride.

Community Input

Dentists, advocates, researchers, and concerned members of the public shared their views with the panel.



Consensus Statements

DOH staff drafted potential consensus statements

Panel reviewed, discussed, edited

Vote in Zoom chat from 1-5

Votes of 4, 5 considered consensus

More discussion and another round of voting until consensus reached

Oral health and fluoride

- Oral Health is essential for overall health and well-being, with connections to quality of life, self-esteem, employment, and school and learning.
- Fluoride is an effective tool in preventing tooth decay.
- Dental decay is a preventable disease. Health behaviors related to a combination of diet, oral hygiene, use of fluorides, and regular dental care are key factors. Health education and other public health interventions designed to improve these health behaviors are important for good oral health.

Oral health and fluoride

- When properly used, topical application of fluoride to teeth, including at low levels in saliva, and at higher levels from fluoridated toothpaste, varnishes, and professional fluoride treatments is clearly beneficial to teeth and helps to prevent dental decay.
- Systemic effects of fluoride include both dose-dependent benefits and harms.
- The burden of dental decay is inequitably distributed due to economic and social inequities and lack of access to dental care.

Benefits of Community Water Fluoridation

- Community Water Fluoridation began in the US in 1945 in Grand Rapids, Michigan. Dental decay among school aged children was greatly reduced in the fluoridated area. This led to the expansion of community water fluoridation throughout most of the U.S. As a result, in 2000, the CDC named community water fluoridation as one of the top ten greatest public health interventions from the twentieth century.
- Community Water Fluoridation is an effective tool in the prevention of tooth decay.
- Since about 1975, access to fluoride in consumer dental products such as toothpastes and rinses has become more widespread.

Benefits of Community Water Fluoridation

- As more of the population has access to fluoride in consumer dental products, the magnitude of the added preventive benefit of community water fluoridation for dental decay is lower, as compared to before 1975 when community water fluoridation was the primary way for most people to be exposed to fluoride.
- Some communities that have stopped community water fluoridation have seen increases in dental decay in their communities. The way researchers have measured those increases and the magnitude of these increases has varied.
- The issue of the added benefits of community water fluoridation to reducing oral health inequities is unresolved. Worsening oral health inequities should community water fluoridation be discontinued would be a concern.

Risks of Community Water Fluoridation

- In risk assessment, it is typical to have a margin of safety between the level we know to be harmful and the level people are exposed to. This margin accounts for uncertainties and is usually protective of susceptible or vulnerable populations.
- Optimally fluoridated drinking water can increase the risk of mild, cosmetic dental fluorosis.
- In 2024, the National Toxicology Program found with moderate confidence, that higher estimated fluoride exposures (e.g., as in approximations of exposure such as drinking water fluoride concentrations that exceed the World Health Organization Guidelines for Drinking-water Quality of 1.5 mg/L of fluoride) are consistently associated with lower IQ in children.

Risks of Community Water Fluoridation

- The primary populations of concern for neurodevelopmental risk from fluoride exposure are pregnant people and infants. Developing fetuses and infants are known to be particularly vulnerable to neurodevelopmental hazards.
- Higher fluoride exposure results in more serious health effects. The science is not clear on whether there is a threshold, below which there are no neurodevelopmental risks in vulnerable populations.
- Some people may be getting too much fluoride. The risks of fluoride come from the total amount consumed from a combination of sources, including water, food, black tea and fluoridated dental products.

MCL

- Naturally occurring high levels of fluoride in drinking water have been linked to skeletal and dental fluorosis.
- Under the federal Safe Drinking Water Act, a Maximum Contaminant Level (MCL) is the highest allowable concentration of a contaminant in drinking water.
- Two health hazards are the basis for the primary and secondary maximum contaminant levels (MCL) for fluoride recognized by the Environmental Protection Agency: the primary MCL of 4 mg/L was established to protect against skeletal fluorosis and the secondary MCL of 2 mg/L protects against dental fluorosis.

MCL

- Neurodevelopmental effects are associated with fluoride drinking water levels between 1.5 mg/L and the current MCLs of 4 mg/L and 2 mg/L.
- In February 2024, the EPA Office of Water calculated a new potential MCL (Goal) of 0.9 mg/L to protect against dental fluorosis. They used revised exposure metrics for 1 to <11 years of age because that life stage was identified as a potential critical window of exposure in the development of primary and secondary teeth. This MCL has not yet been formally proposed.

Next Steps

- Future risk/benefit analyses on community water fluoridation should carefully weigh potential neurodevelopmental hazards to vulnerable populations alongside the oral health benefit attributed to the intervention.
- More research is needed to better understand potential neurodevelopmental risks from community water fluoridation at current recommended levels, 0.7 mg/L.
- Additional research on the contribution of community water fluoridation to reducing oral health inequities is needed.
- The science surrounding fluoride and toxicity continues to evolve and should be monitored.

Conclusions

- Community water fluoridation is an effective tool to prevent tooth decay.
- As more of the population has access to fluoride in consumer dental products, the benefit of community water fluoridation for dental decay is smaller. Before 1975, community water fluoridation was the primary way people were exposed to fluoride.
- Some communities that have stopped community water fluoridation have seen increases in dental decay.
- More research is needed on the impact of community water fluoridation on reducing oral health inequities.

Conclusions

- In 2024, the National Toxicology Program found with moderate confidence, that higher estimated fluoride exposures (exposures equivalent to drinking water fluoride concentrations of 1.5 mg/L or higher) are consistently associated with lower IQ in children.
- Pregnant people and infants are the primary populations of concern for neurodevelopmental risk from fluoride exposure. Developing fetuses, and infants are particularly vulnerable to neurodevelopmental hazards.
- More research is needed on the potential neurodevelopmental risks from community water fluoridation at current recommended levels (0.7 mg/L).

Summary

The panel is:

SURE that fluoride prevents tooth decay.

LESS SURE that community water fluoridation contributes a significant added oral health benefit beyond other common exposures to fluoride.

LESS SURE that community water fluoridation has an impact on oral health inequities.

MODERATELY SURE that exposure to higher levels of fluoride coming from a combination of sources poses an IQ risk to developing fetuses and babies.

LESS SURE that optimally fluoridated water poses an IQ risk for developing fetuses and babies in today's environment that has additional sources of fluoride.



Recommendations

The State Board of Health should:

- Keep the current optimal level of fluoride concentration for now. Community water fluoridation should remain a local decision. Communities should carefully weigh the benefits and risks of water fluoridation.
- Begin the rulemaking process to consider adopting a State Action Level of 1.5 mg/L for fluoride.
- Coordinate with the Department of Health and public health partners to update messaging on fluoride to include guidance to limit fluoride exposure for pregnant people, fetuses, and infants.

Next steps

- Ethics Review
- Prepare for an uncertain future



Acknowledgments: Science Review Panel

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SBOH Authority and Considerations

Shay Bauman, Policy Advisor

Molly Dinardo, Policy Advisor

August 20, 2025

WASHINGTON STATE BOARD OF HEALTH

Washington State

In Washington State, decisions about whether to fluoridate public water systems are made at the local level.

RCW 70.05.060(3) authorizes local boards of health to enact rules necessary to preserve and improve public health, including whether to require fluoridation of public water systems within their jurisdiction (AGO 2008 No.5).

RCW 57.08.012 authorizes fluoridation of a water district's water by a majority vote of water district commissioners.

RCW 43.20.050 grants the Board authority to adopt rules for Group A Public Water Systems necessary to assure safe and reliable drinking water and protect the public health.

- Sets the optimal level for Group A water systems that choose to fluoridate
- Adopts the Maximum Contaminant Level (MCL) to align with federal standards.

Federal Government

EPA: Sets enforceable maximum contaminant levels under the safe drinking water act.

USPHS: Recommends the optimal fluoride level; provides non-binding guidance.

FDA: Regulates fluoride in consumer products.

USDA: Tracks fluoride levels in food and beverages.



Previous Board Recommendations

In an April 2015 Workshop Report, the Board recommended strategies to improve the oral health of Washington residents, including the following:

- Health Systems
- Community Water Fluoridation
- Sealant Programs
- Interprofessional Collaboration
- Oral Health Literacy
- Surveillance
- Workforce Development



Community Water Fluoridation

From the Report:

Expand and Maintain Access to community water fluoridation for the health benefit of children, adults, and seniors

- Support communities that currently provide optimal levels of fluoride to their residents and those seeking to adopt community water fluoridation.

- Support efforts to educate and inform Washington state residents about the importance of fluoridation to improve community health.

- Engage with organizations, agencies and coalitions to promote community water fluoridation in Washington state.



Rulemaking Considerations

Panel consensus statement: Neurodevelopmental effects are associated with fluoride drinking water levels between 1.5 mg/L and the current MCLs of 4 mg/L and 2 mg/L.

Sections of Chapter 246-290 WAC relevant to the panel's recommendation are currently open for rulemaking to address PFAS.

Recommendations

- Remove the *Recommended Strategies to Improve the Oral Health of Washington Residents* (2015) document and accompanying webpage from the Board's website.
    - Work with the Department of Health to review and update the document's content and messaging.
- Direct staff to brief the Board at a future meeting regarding a potential rulemaking to consider adopting a SAL or state MCL for fluoride.

WASHINGTON STATE BOARD OF HEALTH

THANK YOU

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- Our agency, website, and online services follow the Americans with Disabilities (ADA) standards, Section 508 of the Rehabilitation Act of 1973, Washington State Policy 188, and Web Content Accessibility Guidelines (WCAG) 2.0, level AA. We regularly monitor for compliance and invite our users to submit a request if they need additional assistance or would like to notify us of issues to improve accessibility.
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    - The URL (web address) of the content you would like to access
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WASHINGTON STATE DEPARTMENT OF HEALTH

Report of the Fluoride Science Review Panel, August 2025



Washington State Department of HEALTH

DOH 333-397 July 2025

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Executive Summary

Situation

In 2024, the State Board of Health received multiple petitions for rulemaking regarding fluoride exposure for pregnant people, infants, and children. Included were recommendations against adding chemicals such as fluoride to drinking water to treat or prevent disease in humans or animals. The petitions cited findings from the National Toxicology Program (NTP) showing an association between high levels of fluoride exposure and lowered IQ in children. The Department of Health convened a panel of public health staff to review the science and advise the State Board of Health.

Background

The panel's job was to summarize their learnings and interpret the science so that the State Board of Health could consider it in potential policy action. The panel narrowly focused on the risks and benefits of community water fluoridation. We did not assess the evidence around prevention of tooth decay, other forms of fluoride use or exposure, or healthy brain development.

Assessment

Department of Health staff presented the National Toxicology Program monograph, a recent court finding involving the Environmental Protection Agency (EPA) and the Toxic Substances Control Act (TSCA), a Cochrane review, and several additional studies. Additionally, the panel heard from a Department of Health economist, the author of several of the studies reviewed by the NTP, the Arcora Foundation, and other expert and volunteer community members.

Community water fluoridation was very effective when it began in 1945. Many health organizations continue to recommend it. The Cochrane Review raises questions about the added value of community water fluoridation today, given other modern fluoride sources. The NTP reported with moderate confidence that higher estimated fluoride exposures are consistently associated with lower IQ in children. High concentrations of fluoride are also toxic to bones to tooth enamel.

For many people, the oral health benefits of community water fluoridation at current levels may outweigh the risks. However, pregnant people and infants should be considered separately for two reasons. Firstly, they tend to drink more water for their body size than other demographic and age groups. Additionally, developing fetuses, infants and young children are particularly vulnerable to neurodevelopmental hazards.

Recommendations

The State Board of Health should:

- Keep the current optimal level of fluoride concentration for now. Community water fluoridation should remain a local decision. Communities should carefully weigh the benefits and risks of water fluoridation.
- Begin the rulemaking process to consider adopting a State Action Level of 1.5 mg/L for fluoride.
- Coordinate with the Department of Health and public health partners to update messaging on fluoride to include guidance to limit fluoride exposure for pregnant people, fetuses, and infants.

Context

Current Situation

In Washington, the State Board of Health sets an optimal level of fluoridation for water systems that choose to provide fluoridated water. In 2016, the State Board of Health set the optimal level of fluoride at 0.7 mg/L.

Washington state law (RCW 57.08.012) allows, but does not require, community water fluoridation. Local governments make the decision of whether to provide optimally fluoridated water to their communities. Who the local government decision-making authority is varies depending on local governance structure and whether the water system is publicly or privately operated. The decision to optimally fluoridate community water can be controversial.

In 2024, the State Board of Health received multiple petitions for rulemaking regarding fluoride exposure for pregnant people, infants, and children, and recommendations against adding chemicals such as fluoride to drinking water to treat or prevent disease in humans or animals. The petitions cited findings reported by the National Toxicology Program. The Department of Health (DOH) convened a panel to review the science and advise the State Board of Health.

Background

Fluoride is a naturally occurring mineral. Today it is present in drinking water, food, and consumer products. Fluoride has been widely promoted for oral health benefits. Oral health interventions include community water fluoridation, fluoridated toothpaste, and fluoride varnishes. At high concentrations in water, fluoride is toxic to bones (4 mg/L) and tooth enamel (2 mg/L). There are also emerging concerns that fluoride may negatively impact neurodevelopment.

Community water fluoridation began in 1945 in Grand Rapids, Michigan. It resulted in dramatic declines in dental decay in school children. Since then, community water fluoridation has been adopted by communities across the country. Before community water fluoridation, tooth decay was widespread and severe. After fluoride was added to the water supply in many places in the U.S., tooth decay declined in both children and adults. Complete tooth loss in older adults became rarer. In 2000, the Centers for Disease Control and Prevention (CDC) named community water fluoridation as one of the top ten public health successes of the 1900s. Indeed, there is little debate that community water fluoridation was very effective at preventing dental decay prior to about 1975, when fluoride became more common in consumer products like toothpaste.

In Washington, 64% of the population drinks optimally fluoridated water provided by a public water system.

DOH works on health issues relating to water fluoridation and the health of pregnant people, infants, and children in the following ways:

Office of Drinking Water:

- Provides technical assistance to water systems that decide to optimally fluoridate their water.
- Ensures that naturally occurring fluoride in the water stays below the EPA's maximum contaminant level (MCL).

Office of Healthy and Safe Communities, State Oral Health Program:

- This program works to promote and facilitate policies, systems, and partnerships that:
    - Increase the awareness of relationships between oral health and systemic health.
    - Prevent or reduce oral/craniofacial disease and injuries of the head, neck, and oral cavity.
    - Improve access/reduce barriers to preventive oral health services and dental care.
- Promotes strategies that protect oral health, including the benefits of fluoride for the prevention and management of dental decay.
- Occasionally provides technical assistance to communities about community water fluoridation.

Prevention and Community Health Division:

- Works to prevent disease and promote a healthy start, healthy choices, and access to services for children and families, including during pregnancy.
- Provides nutrition education and programs, including the Special Supplemental Nutrition Program for Women and Infants, also known as WIC.

Controversy

Community water fluoridation has been controversial since it began. Some residents of Grand Rapids complained of adverse health effects due to fluoride before the intervention even started.

People who oppose community water fluoridation generally do so based on:

- Concerns for public safety
- The value for bodily autonomy
- Concerns about the proper role of government.

People who support community water fluoridation generally do so based on:

- The long history of apparently safe water fluoridation in the U.S.
- The belief that community water fluoridation prevents dental decay
- The value for equitable public health approaches to disease prevention that do not depend on access to care or other resources.

Science Review Panel

Participants

Panel participants represented the State Board of Health, the State Department of Health, Local Health Jurisdictions, and Tribal Health Organizations. Staff of these agencies were invited to listen to the panel proceedings. They also provided technical information to the panel.

A community member expressed concern to the board that the panelists would have pre-formed opinions about community water fluoridation and would be unwilling to fully review the evidence. This community member was invited to observe the panel's work and participate in the panel's Community Input session. As the meetings went on, other observers also joined.

Panel co-chairs:

Lauren Jenks, Assistant Secretary for Environmental Public Health
Dr. Tao Sheng Kwan-Gett, State Health Officer at the Department of Health

Panel participants:

Amber Arndt, Department of Health, Tribal Policy Director
Dr. Allison Berry, Local Health Officer
Shay Bauman, State Board of Health
Dr. Emerson Christie, Department of Health, Toxicologist
Derrick Dennis, Department of Health, Drinking Water
Molly Dinardo, State Board of Health
Dr. Herbie Duber, Department of Health, Regional Medical Officer
Phuc Ha, Local Public Health
Lindsay Herendeen, State Board of Health
Lauren Jenks, Department of Health, Environmental Public Health
Dr. Tao Sheng Kwan-Gett, Department of Health, State Health Officer
Dr. Tom Locke, Tribal Health Officer
Dr. Bob Lutz, Local Health Officer
Shawn Magee, Local Public Health
Dr. Jessica Marcinkevage, Department of Health, State Epidemiologist for Policy and Practice
Dr. Kari Mentzer, Department of Health, Epidemiologist
Michele Roberts, Department of Health, Prevention and Community Health

Charge to the Panel

The panel was charged with listening, learning, and considering all relevant science in its discussions of community water fluoridation. It was further charged with summarizing its learnings and interpreting the science so that the State Board of Health could consider it for potential policy action.

As the panel worked together, its discussion began to center around how best to get the benefits of community water fluoridation while minimizing the risk of fluoride exposure to fetuses and infants.

Process

The panel met 10 times from January through June 2025. The meetings were held over Zoom and generally lasted about 2 hours. The meeting format was approximately 1 to 1.5 hours of presentation followed by discussion and questions. When possible, questions were answered during the same

meeting. If more research was required, questions were answered in the next meeting. The slides presented at the meetings are available in Attachment A.

Based on the science and panel discussions, DOH staff drafted potential consensus statements ahead of the meeting. The panel reviewed the draft consensus statements and edited them during the meetings. When it looked like we might have gotten the statements to a point of consensus, we voted by putting a number from 1 (completely disagree) to 5 (wholeheartedly agree) in the meeting chat. Fours and fives were generally considered approval of the statements. Anyone who voted lower was asked to explain their concerns. More discussion and another vote would follow. A similar process was followed to develop the conclusions and recommendations. Not all panelists attended all voting sessions, and an individual panelist's views may differ from this report.

Limitations

The panel narrowly focused on the risks and benefits of community water fluoridation. We did not assess the evidence around other strategies for the prevention of tooth decay. We also did not assess the evidence around healthy brain development or measurement of IQ in children. Occasionally, we heard information related to potentially less risky methods of getting the benefits of fluoride and other health behaviors that improve oral health. We did not review the evidence around these interventions. Changes to the health care system or dental care system were also out of the panel's scope.

Summary of Information Reviewed at Each Meeting

This is a summary of the information that was reviewed at the meetings. This summary is not a comprehensive review of the literature or a critical review of what was presented.

Pre-work	Review Dr. Kyla Taylor's explanation of the NTP monograph on a Collaborative for Health and the Environment webinar.

Meeting 1
January 9
	Dr. Emerson Christie, Department of Health, summarized *Fluoride, Neurodevelopment, and Cognition: A National Toxicology Program Monograph*.
	• Moderate confidence that higher estimated fluoride exposures are consistently associated with lower IQ in children.
	• Unsure: whether low fluoride level of 0.7 mg/L currently recommended for US community water has a negative effect on children's IQ.
	• No evidence: that fluoride exposure has adverse effects on adult cognition.
	• Strengths: The National Toxicology Program (NTP) Monograph on Fluoride Exposure and Neurodevelopment (August 2024) is a comprehensive assessment of the scientific literature.
	  ○ The monograph identified a large body of evidence (72 epidemiologic studies) evaluating associations between fluoride exposure and IQ in children. Of the 19 high-quality studies, 18 found a relationship between higher fluoride and lower IQ in children.
	  ○ The high-quality studies included 3 prospective cohort studies and 15 cross-sectional studies spanning 5 different countries (none in the U.S.). Of the remaining 53 studies,

	46 also provided evidence of inverse associations between fluoride exposure and IQ. • NTP also reviewed studies of other neurodevelopmental conditions (e.g. memory, ADHD) and identified 8 out of 9 high-quality studies observed inverse associations with fluoride exposure. • Limitations: The database on toxicity is not complete (EPA 2024).
Meeting 2 January 28	Dr. Emerson Christie, Dr. Holly Davies, and Michael Ellsworth, JD, Department of Health, summarized the 2024 EPA court judgement on fluoride and the Toxic Substances Control Act. • In November 2016, a group of organizations and individuals petitioned the EPA under Section 21 of Amended Toxic Substances Controlled Act to regulate the fluoridation of drinking water supplies under Section 6(a). • They alleged that fluoridation at levels occurring throughout the country presented an unreasonable risk of injury to health under the Toxic Substances Control Act ("TSCA"), 15 U.S.C. § 2620(b)(4)(B). • After the EPA denied the petition, in April 2017, the organizations and individuals filed suit seeking judicial review of the EPA's denial pursuant to 15 U.S.C. § 2620. • For a risk to be present, the court must find that some segment of the United States population is exposed to fluoride in drinking water at levels that either exceed or are too close to the dosage at which fluoride presents a hazard. • The Court found that fluoridation of water at 0.7 mg/L poses an unreasonable risk of reduced IQ in children. • This does not mean that the court found that fluoridated water is definitely harmful. Rather, the court found an unreasonable risk of harm, which is a toxicological standard used by EPA under TSCA. • EPA argued that there is uncertainty around the hazard level and the precise relationship between dosage and response at lower levels. The court found these arguments "not persuasive" because of the requirement for a margin of safety between the hazard level and the dose. Even accounting for the uncertainty, that margin of safety was not currently met. • EPA argued that maternal urinary fluoride is not a good way to measure effects of community water fluoridation because it will include all exposures to fluoride—including toothpaste, mouthwash, etc. The court found that EPA must consider the additive effect of all exposures to fluoride to adequately assess safety. • The court did not consider the benefits of fluoride in their review. • A court finding is not a scientific finding. It is an interpretation of the science that exists in reference to current federal law.

Meeting 3 February 11	Shelley Guinn, Department of Health, reviewed the causes of tooth decay, the burden of disease and inequities, relative efficacy and mechanism of action of different fluoride applications, the modes of fluoride intake, and public health and economic considerations of community water fluoridation. Arcora and Health Care Authority discussed access to dental health care in Washington. • Oral health impacts physical and mental health, school and work attendance, and many aspects of quality of life. • Exposure to fluoride hardens the tooth enamel and is protective against tooth decay in children and adults. • Community water fluoridation is a long-standing intervention intended to provide the benefits of fluoride and good oral health to large portions of the community, regardless of access to dental care or fluoride-containing hygiene products. • Access to dental care varies by employment and income and location in the state. Many children and adults in the state lack adequate access to dental care.
Meeting 4 March 11	Claire Nitcshe and Dr. Kyle Yomogida, Department of Health, reviewed the 2024 Cochrane Review, *Water fluoridation for the prevention of dental caries*. • Studies conducted in 1975 and earlier showed a clear and beneficial effect of community water fluoridation on prevention of tooth decay in children. However, due to the increased availability of fluoride in toothpaste since 1975, we do not see the same size effect at a population level today. • Studies conducted after 1975 showed that adding fluoride to water may lead to slightly less tooth decay in children's baby teeth. • Adding fluoride to water may slightly increase the number of children who have no tooth decay in either their baby teeth or permanent teeth. However, these results also included the possibility of little or no difference in tooth decay. • Unsure: whether adding fluoride to water reduced tooth decay in children's permanent teeth. • Unsure: whether there are any effects on tooth decay when fluoride is removed from a water supply. • Unsure: whether fluoride reduces differences in tooth decay between people with higher incomes and people with lower incomes. • The authors point out that a finding of insufficient evidence of an effect is not the same as evidence of no effect. • The panel hypothesized that additional studies on the effects of community water fluoridation on health disparities may find that community water fluoridation reduces oral health disparities.
Meeting 5 March 25	Dr. Herbie Duber, Department of Health, reviewed additional information on tooth decay: burden of the disease, cumulative all-cause mortality as related to tooth decay, and oral disorders costs. Dr. Duber reviewed two

	case studies: Calgary, Alberta, ("Community water fluoride cessation and rate of caries-related pediatric dental treatments under general anesthesia in Alberta, Canada" and "Equity in children's dental caries before and after cessation of community water fluoridation") and Juneau, Alaska ("Consequences of community water fluoridation cessation for Medicaid-eligible children and adolescents in Juneau, Alaska"). • There are strong associations between dental caries and stroke and all-cause mortality. • Oral disorder costs make up about 3.8% of US health care spending, with an estimated cost of $93 billion. • Community water fluoridation was very effective for the prevention of tooth decay when it began in 1945, and it continues to be recommended by many health organizations including the American Dental Association and the American Academy of Pediatrics. • Cochrane Review raises questions about the added value of community water fluoridation given alternative fluoride sources in modern society. • Calgary, Canada: Fluoride was introduced to drinking water in 1991, removed in 2011, and reintroduced in 2021. Discontinuing community water fluoridation was associated with increased dental treatment under general anesthesia, especially among children 0-5 years old. • Calgary, Canada: Odds of untreated dental decay increased more among those without dental insurance from 2009/2010 to 2013/2014, showing an increase in disparity. The authors present multiple possible causes, one of which is the end of community water fluoridation. • Juneau, Alaska: After stopping community water fluoridation, they found significant differences in the mean number of Medicaid eligible dental procedures among 0-6, 0-7, and 0-18 groups, but not significant in ages 7-13 or 13-18. This indicates a significant difference in treatment of primary teeth but not permanent teeth. • Increased dental care costs were correlated with the stopping of community water fluoridation. Anna Hidle, Department of Health, reviewed and presented learnings from economic evidence about community water fluoridation. • Even with the decline of benefits from community water fluoridation, literature continues to report it as a cost-effective intervention. • However, two recent papers call for inclusion of the costs of treating fluorosis and/or lost IQ points which demonstrate a reduction in the historical return on investment.

	• Future economic modeling should consider learnings from lost IQ points due to elevated blood lead levels, including level of impact from exposure and racial disparities.
Meeting 6 April 8	Dr. Charlotte Lewis, Dr. Donald Chi, Dr. Bill Osmunson, Dr. Scott Tomar, and Lauren Johnson provided perspective and comment. • Summarized below under "Community Input"
Meeting 7 April 22	Panel created consensus statements. (See below.)
Meeting 8 May 6	Dr. Christine Till, York University, Toronto, presented an overview of the emerging science on fluoride toxicology and her findings and conclusions from several studies included in the NTP report. • In addition to dental fluorosis, evidence is strong for reduction in IQ scores in children, moderate for thyroid dysfunction, weak for kidney dysfunction, and limited for sex hormone disruptions. • A 1 mg/L increase in fluoride intake was associated with a 3.66 (95% CI,-7.16 to -0.15; p=.04) lower IQ score in boys and girls. • Formula-fed babies are at risk of lower IQ if their formula is made with fluoridated water. • IQ decreases with increasing levels of fluoride in water and urine. There is no obvious threshold. • Evidence of fluoride neurotoxicity at urine fluoride levels <1.5 mg/L is relevant to community water fluoridation because pregnant women and children can exceed an equivalent dose of fluoride even when drinking optimally fluoridated water depending on amount of fluoridated water they ingest and their exposure to other sources of fluoride. • Iodine deficiency may increase the risk of fluoride neurotoxicity. • Certain genetic factors may increase sensitivity to fluoride exposure. • Higher water fluoride, urine fluoride, and serum fluoride concentrations were associated with higher Thyroid Stimulating Hormone (TSH) in children. • Till stated, "Given that fluoride offers little benefit to the fetus and young infant, community-wide administration of systemic fluoride may pose an unfavorable risk-benefit ratio for the pregnant woman, fetus, and infant."
Meeting 9 May 20	Panel created consensus statements and recommendations. (See below.)
Meeting 10 June 17	Panel finalized recommendations to the Board. (See below.)

Summary of Information Reviewed

This section summarizes the information reviewed in the panel meetings. It draws from the meeting summaries above but is organized by topic instead of by date. It is not a comprehensive review of the literature or a critical review of the information presented.

Evidence of Benefits

Fluoride was first added to drinking water in 1945 to help prevent cavities at a population level. At that time, it was very effective. Many health organizations continue to recommend it.

Studies done in 1975 and earlier showed a clear effect of community water fluoridation on prevention of tooth decay in children. After 1975, the benefits may be smaller because fluoride is now found in toothpaste and other products.

In Calgary, Canada, fluoride was added to drinking water in 1991, removed in 2011, and added again in 2021. When fluoride was stopped, more people needed dental surgery under general anesthesia. This was especially true for children 0-5 years old. In the period without fluoridation, the odds of having untreated dental decay increased, with a sharper increase among people without dental insurance.

In Juneau, Alaska, after fluoridation was stopped, young children on Medicaid suffered a significant increase in their average (mean) number of dental decay procedures. Children born after fluoridation ended had the most procedures and the highest treatment costs.

Using the Cochrane method for systematic reviews, Iheozor-Ejiofor, et al., identified 157 studies on fluoridation. They evaluated the effects of starting or stopping community water fluoridation. They also looked at the association of fluoride in drinking water with fluorosis. Twenty-one studies looked at starting water fluoridation. One looked at stopping water fluoridation. All the studies used non-randomized designs. Studies done after 1975 showed that adding fluoride to water may lead to less tooth decay in children's baby teeth and a increase in the number of children who have no tooth decay in either their baby teeth or permanent teeth. However, these results also included the possibility of little or no difference in tooth decay.

This review raises questions about the added value of community water fluoridation given alternative fluoride sources in modern society.

Four dentists spoke to the panel. Several of the dentists reminded the panel that community water fluoridation prevents tooth decay and is endorsed by:

- The Centers for Disease Control and Prevention
- American Medical Association
- American Academy of Pediatrics
- American Dental Association
- American Academy of Pediatric Dentistry
- American Association for Dental, Oral, and Craniofacial Research.

Several of the dentists stated that community water fluoridation remains a safe, cost-effective, and equitable strategy for preventing dental decay in all age groups. More of the dentists' comments can be found in the community input section of this report.

Evidence of Harms

High levels of naturally occurring fluoride can cause skeletal and dental fluorosis. Optimal fluoride levels used in community water are lower and usually cause at most mild, cosmetic dental fluorosis.

A recent systematic review of international studies has shown that higher levels of fluoride in drinking water may be linked to reduced IQ scores in children. There is limited evidence for association with thyroid dysfunction, kidney dysfunction, and sex hormone disruptions. The authors suggest 1.56mg/L of fluoride as a starting point for setting a health-based limit for fluoride in drinking water.

Other studies (mostly in Asia) found that higher water fluoride, maternal urine fluoride, and maternal serum fluoride concentrations are associated with impacts on thyroid function and an increased risk of some thyroid diseases.

A Canadian study found that higher levels of fluoride exposure during pregnancy were associated with lower IQ scores in children when they were 3-4 years old. A systematic review and meta-analysis found a dose-response associate between increasing levels of fluoride in water and urine and lower IQ in children. Another Canadian study found that babies who drink formula mixed with fluoridated water may also be at risk of lower IQ.

Many studies of IQ and fluoride have been done with water at high levels of fluoride (about 1.5 mg/L). This is higher than what is normally found in U.S. drinking water. However, some studies have been done with water fluoride concentrations or maternal urinary fluoride concentrations comparable to those in the U.S. Additionally, because pregnant women and children drink more water for their size, they can end up having exposures similar to people in places with higher levels of water fluoridation. Factors like iodine deficiency and certain genes may also make some people more sensitive to fluoride.

Till et all stated, “Given that fluoride offers little benefit to the fetus and young infant, community-wide administration of systemic fluoride may pose an unfavorable risk-benefit ratio for the pregnant woman, fetus, and infant.”

In 2024, the National Toxicology Program looked at 72 studies about fluoride and IQ. Of the 19 high-quality studies, 18 found a relationship between higher fluoride and lower IQ in children. The high-quality studies included 3 prospective cohort studies and 15 cross-sectional studies spanning 5 different countries (none in the U.S.). Of the remaining 53 studies, 46 also provide evidence of inverse associations between fluoride exposure and IQ. They also reviewed studies of other neurodevelopmental conditions (e.g. memory, ADHD). Eight out of 9 high-quality studies observed inverse associations with fluoride exposure.

The National Toxicology Program concluded with moderate confidence that higher estimated fluoride exposures are consistently associated with lower IQ in children. These levels of exposure are approximately equivalent to 1.5 mg/L of fluoride or more in drinking water. However, there wasn’t enough evidence to say whether lower levels, like the 0.7 mg/L used in the U.S., have the same effect. They found no evidence that fluoride exposure harms adult brain function. The EPA has noted that we are still learning about fluoride toxicity.

Economic Analysis

Most studies about fluoride and economic costs focus on:

- Saving on dental care costs.
- Avoiding lost work time.
- How much it costs water systems to add fluoride.

Most studies do not count the cost of treating fluorosis or possible IQ loss.

Authors of a cost-effectiveness analysis , used data for 8484 children (mean age 9.6 years) from the 2013-2016 NHANES to create a model that they then used to simulate the impacts of stopping community water fluoridation. The model projected that stopping community water fluoridation in the U.S. could cause a 7.5% increase in tooth decay and cost approximately $9.8 billion over 5 years. Children who are uninsured or publicly insured would be disproportionately affected compared to those with private dental insurance. Sensitivity analyses using lower efficacy estimates from fluoridation found lower, but still substantial, harms.

Recent literature continues to report community water fluoridation is a cost-effective intervention, though its return on investment is lower than it used to be.

Community Input

Dentists, advocates, researchers, and concerned members of the public volunteered to share their views with the panel. We heard summaries of fluoride toxicity and the efficacy of community water fluoridation. Different people reached different conclusions based on the science. Some told painful personal stories of sensitivity to fluoride. We also heard powerful endorsements of community water fluoridation. People passionately expressed deeply held values that inform their opinion on community water fluoridation.

Comments regarding the benefits of community water fluoridation included:

- Community water fluoridation prevents tooth decay (Cochrane reported this with Low Certainty). Does not prevent tooth decay as much as before 1975, but it still prevents some.
- Mistrust of NTP report because of lack of awareness of any reliable study of IQ in 3-4 year-olds
- US Preventive Services Task Force gives fluoride supplements for children 6 months to 5 years old a B rating.
- Community water fluoridation is endorsed by CDC, American Medical Association, American Academy of Pediatrics, American Dental Association, American Academy of Pediatric Dentistry, American Association for Dental, Oral, and Craniofacial Research
- Community water fluoridation is a population-based intervention that helps everyone, especially people at high risk for tooth decay.
- If sugar wasn't a problem, community water fluoridation wouldn't be as important.
- Fluoride is not a chemical, it's a natural substance.
- Hardwick, 1982: community water fluoridation resulted in 26.8% fewer cavities
- Blinkhorn, 2015: community water fluoridation resulted in 32.3% fewer cavities
- Goodwin, 2022: community water fluoridation resulted in 26% fewer cavities in primary teeth and 13% in permanent teeth
- There is no association between community water fluoridation and changes in IQ.

- Community water fluoridation still prevents dental decay even with widespread use of fluoride toothpaste, though smaller absolute effect than we once saw.
- Recent studies suggest cessation of community water fluoridation increases dental decay and costs in children
- No other developed country has changed fluoridation policy due to NTP report or EPA court case, and UK recently announced plan to expand fluoridation.
- Cross-sectional studies consistently show fewer cavities in fluoridated communities, although less so than in the 1950's.
- As feds cut Medicaid, community water fluoridation will be more important for high-risk communities.
- 2020 North Carolina study found that lifetime community water fluoridation exposure (through age 19) virtually eliminated oral health socioeconomic disparities.
- Despite the influence of political forces, community water fluoridation remains a safe, cost-effective, and equitable strategy for prevention of dental decay for individuals of all ages.
- Epidemiologic evidence informs our understanding that fluoride is beneficial throughout the lifespan.

Comments bringing concerns about community water fluoridation included:

- The State Board of Health has a key responsibility to ensure safe drinking water. This responsibility is more important than providing for the marginal benefits to health from community water fluoridation.
- Fluoride is not good for everyone. Some people experience unique toxic effects. Some people are extremely sensitive to exposure to fluoride.
- Putting fluoride in water for a specific health effect is treating fluoride like a drug, but it has not been approved as a drug. Not everyone has consented to receiving this drug.
- Fluoride in the water is not effective at preventing cavities, but it does present an unacceptable neurodevelopmental risk.
- We should make decisions following the precautionary principle and not expose people to a chemical we cannot prove is safe for everyone.
- Washington's code regarding community water fluoridation has not kept up with the science and must be updated.
- Other states are beginning to question or ban community water fluoridation.
- IQ impacts are more important to consider than oral health impacts because a cavity can be filled but a loss of IQ points are forever.
- People should be able to choose whether they have fluoride in their water. People should be able to opt out of fluoride if they don't want it.
- Disease prevalence rates are dynamic. We may be inappropriately attributing changes in the rates of dental decay to community water fluoridation.
- Too much exposure to fluoride also exposes teeth to risk of decay through fluorosis.
- The certainty with which presenters state "community water fluoridation prevents tooth decay" is more marketing than science.
- There is no reason for babies under 6 months of age to get any fluoride. This is consistent with the US Preventive Services Task Force recommendations.

- Endorsements are not science.
- Community Water Fluoridation takes away freedom
- Concentration in drinking water is not the same as dose—dose may be too high for fetuses and infants.
- Most European dental associations no longer recommend fluoride supplements.
- Comparing 50 states, fluoridation is not associated with better dental health.
- Effectiveness of community water fluoridation has declined over time.

Consensus Statements

The following are consensus statements agreed upon by the panel after reviewing and discussing the scientific and community input.

Oral Health and Fluoride

1. Oral Health is essential for overall health and well-being, with connections to quality of life, self-esteem, employment, and school and learning.
2. Fluoride is an effective tool in preventing tooth decay.
3. Dental decay is a preventable disease. Health behaviors related to a combination of diet, oral hygiene, use of fluorides, and regular dental care are key factors. Health education and other public health interventions designed to improve these health behaviors are important for good oral health.
4. When properly used, topical application of fluoride to teeth, including at low levels in saliva, and at higher levels from fluoridated toothpaste, varnishes, and professional fluoride treatments is clearly beneficial to teeth and helps to prevent dental decay.
5. Systemic effects of fluoride include both dose-dependent benefits and harms.
6. The burden of dental decay is inequitably distributed due to economic and social inequities and lack of access to dental care.

Community Water Fluoridation Benefits

1. Community Water Fluoridation began in the US in 1945 in Grand Rapids, Michigan. Dental decay among school aged children was greatly reduced in the fluoridated area. This led to the expansion of community water fluoridation throughout most of the U.S. As a result, in 2000, the Centers for Disease Control and Prevention named community water fluoridation as one of the top ten greatest public health interventions from the twentieth century.
2. Community Water Fluoridation is an effective tool in the prevention of tooth decay.
3. Since about 1975, access to fluoride in consumer dental products such as toothpastes and rinses has become more widespread.
4. As more of the population has access to fluoride in consumer dental products, the magnitude of the added preventive benefit of community water fluoridation for dental decay is lower, as compared to before 1975 when community water fluoridation was the primary way for most people to be exposed to fluoride.
5. Some communities that have stopped community water fluoridation have seen increases in dental decay in their communities. The way researchers have measured those increases and the magnitude of these increases has varied.
6. The issue of the added benefits of community water fluoridation to reducing oral health inequities is unresolved. Worsening oral health inequities should community water fluoridation be discontinued would be a concern.

Community Water Fluoridation Risks

1. In risk assessment, it is typical to have a margin of safety between the level we know to be harmful and the level people are exposed to. This margin accounts for uncertainties and is usually protective of susceptible or vulnerable populations.
2. Optimally fluoridated drinking water can increase the risk of mild, cosmetic dental fluorosis.

3. In 2024, the National Toxicology Program found with moderate confidence, that higher estimated fluoride exposures (e.g., as in approximations of exposure such as drinking water fluoride concentrations that exceed the World Health Organization Guidelines for Drinking-water Quality of 1.5 mg/L of fluoride) are consistently associated with lower IQ in children.
4. The primary populations of concern for neurodevelopmental risk from fluoride exposure are pregnant people and infants. Developing fetuses and infants are known to be particularly vulnerable to neurodevelopmental hazards.
5. Higher fluoride exposure results in more serious health effects. The science is not clear on whether there is a threshold, below which there are no neurodevelopmental risks in vulnerable populations.
6. Some people may be getting too much fluoride. The risks of fluoride come from the total amount consumed from a combination of sources, including water, food, black tea and fluoridated dental products.

Maximum Contaminant Level

1. Naturally occurring high levels of fluoride in drinking water have been linked to skeletal and dental fluorosis.
2. Under the federal Safe Drinking Water Act, a Maximum Contaminant Level (MCL) is the highest allowable concentration of a contaminant in drinking water.
3. Two health hazards are the basis for the primary and secondary maximum contaminant levels (MCL) for fluoride recognized by the Environmental Protection Agency: the primary MCL of 4 mg/L was established to protect against skeletal fluorosis and the secondary MCL of 2 mg/L protects against dental fluorosis.
4. Neurodevelopmental effects are associated with fluoride drinking water levels between 1.5 mg/L and the current MCLs of 4 mg/L and 2 mg/L
5. In February 2024, the EPA Office of Water calculated a new potential MCL (Goal) of 0.9 mg/L to protect against dental fluorosis. They used revised exposure metrics for 1 to <11 years of age because that life stage was identified as a potential critical window of exposure in the development of primary and secondary teeth. This MCLG has not yet been formally proposed.

Next Steps

1. Future risk/benefit analyses on community water fluoridation should carefully weigh potential neurodevelopmental hazards to vulnerable populations alongside the oral health benefit attributed to the intervention.
2. More research is needed to better understand potential neurodevelopmental risks from community water fluoridation at current recommended levels, 0.7 mg/L.
3. Additional research on the contribution of community water fluoridation to reducing oral health inequities is needed.
4. The science surrounding fluoride and toxicity continues to evolve and should be monitored.

Conclusions

- Community water fluoridation is an effective tool to prevent tooth decay.
- As more of the population has access to fluoride in consumer dental products, the benefit of community water fluoridation for dental decay is smaller. Before 1975, community water fluoridation was the primary way people were exposed to fluoride.
- Some communities that have stopped community water fluoridation have seen increases in dental decay.
- More research is needed on the impact of community water fluoridation on reducing oral health inequities.
- In 2024, the National Toxicology Program found with moderate confidence, that higher estimated fluoride exposures (exposures equivalent to drinking water fluoride concentrations of 1.5 mg/L or higher) are consistently associated with lower IQ in children.
- Pregnant people and infants are the primary populations of concern for neurodevelopmental risk from fluoride exposure. Developing fetuses, and infants are particularly vulnerable to neurodevelopmental hazards.
- More research is needed on the potential neurodevelopmental risks from community water fluoridation at current recommended levels (0.7 mg/L).

Summary and Recommendations

The panel summarized their assessment of the science in this way.

We are:

> **SURE** that fluoride prevents tooth decay.
>
> **LESS SURE** that community water fluoridation contributes a significant added oral health benefit beyond other common exposures to fluoride.
>
> **LESS SURE** that community water fluoridation has an impact on oral health inequities.
>
> **MODERATELY SURE** that exposure to higher levels of fluoride coming from a combination of sources poses an IQ risk to developing fetuses and babies.
>
> **LESS SURE** that optimally fluoridated water poses an IQ risk for developing fetuses and babies in today's environment that has additional sources of fluoride.

The panel recognized that the State Board of Health is in the difficult position of considering science-based policy options at a time when the scientific data is inadequate and emerging. There are multiple reasonable pathways available to the Board. Of the available options, the panel recommended that The State Board of Health should:

- Keep the current optimal level of fluoride concentration for now. Community water fluoridation should remain a local decision. Communities should carefully weigh the benefits and risks of water fluoridation.
- Begin the rulemaking process to consider adopting a State Action Level of 1.5 mg/L for fluoride.
- Coordinate with the Department of Health and public health partners to update messaging on fluoride to include guidance to limit fluoride exposure for pregnant people, fetuses, and infants.

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Attachment A: Report from Department of Health Toxicologists

The Washington State Department of Health Site Assessment and Toxicology (SAT) were tasked with evaluating the National Toxicology Program's (NTP) Monograph on Fluoride Exposure and Neurodevelopment and other research related to fluoride and neurodevelopmental toxicity. The summary of key findings below is not intended as a formal risk assessment of fluoride exposure and this work does not represent the derivation of a protective value. However, a risk framework for the information may aid in decision-making.

Hazard Assessment

Several hazards of fluoride ingestion have been identified. The strength of association with fluoride exposure varies for several different hazards. Two health hazards are the basis for the primary and secondary maximum contaminant levels (MCL) recognized by the Environmental Protection Agency (EPA 1986): the primary MCL of 4 mg/L was established to protect against skeletal fluorosis (4 mg/L) and the secondary MCL of 2 mg/L protects against dental fluorosis. Skeletal fluorosis is characterized by pain in the bones and joints and by a weakening of bones resulting in an increase in fractures (NRC 2006). Dental fluorosis is characterized by the discoloration and pitting of the dental enamel (NRC 2006). Less severe dental fluorosis is considered a cosmetic issue (i.e. discoloration), however, more severe forms cause enamel loss (i.e. pitting), which may make teeth more susceptible to decay.

The evidence for fluoride hazards has expanded beyond the effects of skeletal and dental fluorosis to include neurodevelopmental impacts, particularly on children's intelligence quotient (IQ). Over the past two decades, research has increasingly focused on fluoride's potential neurotoxic effects, with IQ being the primary endpoint considered in most studies. IQ is a common metric for evaluating population-level effects of environmental contaminants on cognition (Grandjean and Landrigan 2006, Grandjean and Landrigan 2014) and has proved a useful indicator of adverse effects for many contaminants including lead (ATSDR 2020) and mercury (Grandjean et al 1997, EPA 2001).

The National Toxicology Program (NTP) Monograph on Fluoride Exposure and Neurodevelopment (August 2024) is a comprehensive assessment of the scientific literature. The monograph identified a large body of evidence (72 epidemiologic studies) evaluating associations between fluoride exposure and IQ in children. Of those, 19 studies were identified as high-quality 18 of which reported inverse associations between fluoride exposure and children's IQ. The high-quality studies included three prospective cohort studies and 15 cross-sectional studies spanning five different countries (none in the U.S.). Of the remaining 53 studies 46 also provide evidence of inverse associations between fluoride exposure and IQ. NTP also reviewed studies with other neurodevelopmental endpoints (e.g. memory, ADHD) and identified eight out of nine high-quality studies that observed inverse associations between fluoride exposure and other neurodevelopmental outcomes.

The NTP monograph also identified and reviewed several meta-analyses, which consistently reported inverse associations between fluoride exposure and children's IQ. Meta-analyses are an approach to combine findings from multiple studies to address a research question. The meta-analyses identified by the NTP incorporated many of the IQ studies evaluated above and had variability in methodologies and effect sizes. One meta-analysis, Kumar et al. (2023) found significant inverse associations at higher drinking water concentrations (average 3.7 mg/L) but not at lower concentrations (average 0.9 mg/L).

The ultimate finding from the NTP monograph was that with moderate confidence water fluoride concentrations of 1.5 mg/L or greater are consistently associated with lower IQ in children. They acknowledge that more studies are needed, particularly at lower fluoride concentrations to upgrade the moderate confidence conclusion to high confidence.

Most recently, the NTP authors conducted a meta-analysis (Taylor et al. 2025) that serves as a companion hazard assessment to the NTP report. When restricted to high-quality studies, a significant inverse dose-response relationship was identified between IQ in children and group-level fluoride exposure at drinking water concentrations of 4 mg/L, 2 mg/L, and 1.5 mg/L. A dose-response relationship was also noted when urinary fluoride, rather than drinking water intake, was the exposure metric. Thirteen high-quality studies, each using individual-level measures, revealed a decrease of 1.1 IQ points for every 1 mg/L increase in maternal urinary fluoride.

The results of the NTP monograph and the NTP authors meta-analyses provide evidence that neurodevelopmental effects can occur at fluoride drinking water levels below the current MCLs of 4 mg/L and 2 mg/L. It is important to note that these MCLs were set with no uncertainty factors applied with the rationale at the time provided briefly as follows: no uncertainty due to the oral health benefit, the dental fluorosis endpoint was modeled using epidemiologic data from children and therefore no uncertainty regarding different populations was necessary, and the EPA considered the database for fluoride toxicity to be complete (EPA 2010a). In February of 2024 the EPA Office of Water released a review of health effects for primary drinking water standards where they evaluated recent data for fluoride on dental health effects and exposure. In that report they calculated a new potential MCLG of 0.9 mg/L using revised exposure metrics for 1 to <11 years of age because that life stage was identified as a potential critical window of exposure in the development of primary and secondary teeth (EPA 2024). In the same report they also stated that they are aware of studies reporting an association between fluoride exposure and neurodevelopmental effects in the published literature acknowledging that the database on toxicity is not complete (EPA 2024). Additionally, the EPA has recently announced they intend to perform an updated evaluation of fluoride hazards (EPA 2025).

Exposure Assessment

In risk, exposure is evaluated by both the population exposed and the magnitude. As the neurodevelopment hazard has been related to maternal urinary fluoride concentrations, child urinary fluoride concentrations, drinking water concentration, and infant formula concentrations (NTP 2024) the primary populations of concern are pregnant people, infants, and children. Emphasis is given to pregnant people (i.e. the developing fetus) and infants as these life stages have historically shown vulnerability to neurodevelopmental hazards.

Drinking water concentration has traditionally been used as the primary metric for fluoride exposure in regulatory contexts. Drinking water is generally considered a direct, frequent, and indiscriminate exposure pathway for most populations but the magnitude can be variable depending on consumption. As described above drinking water fluoride concentrations and reduced IQ are associated at concentrations of 1.5 mg/L or greater, although it should be noted that individual studies from Canada have assessed reduced IQ in populations with drinking water at concentrations below 1.5 mg/L (Green et al 2019).

However, drinking water as an exposure metric has several important limitations. Water concentration is not a direct measure of dose but rather requires estimation to determine actual intake as individual water consumption varies significantly. According to EPA estimates, drinking water accounts for 40-70% of total fluoride exposure for most individuals (EPA 2010b). This highlights two important things: 1) drinking water is in most cases the primary route of exposure to fluoride in the U.S. and 2) drinking water, although often the primary route of exposure, is not the only exposure to consider. Integration across all exposure sources and routes provides more useful risk characterization.

Urinary fluoride concentration is more commonly used as a biomarker as it provides an integrated measure of total fluoride exposure across all sources including water, food, and dental products. It has been validated in multiple cohort studies as reliably associated with neurodevelopmental outcomes (Green et al 2019, Bashash et al 2017, Malin et al 2024). Recent research has provided data on fluoride exposure in several areas throughout the U.S. and neighboring countries based upon urinary fluoride. Malin et al. (2024) identified median maternal urinary fluoride levels (MUF) in Los Angeles California of 0.76 mg/L. Griebel-Thompson et al. (2025) performed a multi-state evaluation (IN, KS, KY, MO, and OH) that found baseline median MUF of 1.0 mg/L in fluoridated areas and 0.80 mg/L in non-fluoridated areas.

These US-based findings are corroborated by data from international cohort studies. A Canadian cohort reported mean MUF of 0.51 mg/L, with significantly higher levels (0.69 mg/L) among women living in areas with fluoridated water compared to those in non-fluoridated areas (0.40 mg/L) (Green et al 2019). Also, a Mexican cohort study found mean MUF of 0.9 mg/L, however associated water fluoride levels were not available (Bashash et al 2017). These data suggest that the MUF in the U.S. are comparable to those in neighboring countries, that MUF may be associated with water fluoridation, and that U.S. MUF are at or near those that have been identified by the NTP authors for inverse impact to IQ and exceed the level (0.28 mg/L) put forward by Grandjean et al 2024.

Risk Evaluation

A fundamental tenet of risk assessment is adequately capturing the uncertainty when establishing protective levels. Using the lowest level where an adverse effect was observed or the level where no adverse effect was observed is not necessarily a level that will be protective of public health. Protective levels should provide a buffer against uncertainties in the data and variability in human susceptibility.

Uncertainty factors (UFs) provide a systematic approach to weighing various types of uncertainty in the data. UFs may be assigned for the following sources of uncertainty and variability in the data that are being used to define a protective exposure level: using a lowest observed adverse effect level (LOAEL) instead of a no observed adverse effect level (NOAEL), intraspecies variability, interspecies variability, database uncertainty, and using sub-chronic data in place of chronic data. UFs are typically valued at 3 (half order of magnitude rounded down) and 10 (full order of magnitude) depending on the nature and quality of available data. Multiple UFs may be multiplied together to establish the total margin between observed effect levels and acceptable exposure limits.

As discussed above, the EPA when setting the MCLs elected to set the UF = 1. While this may have been defensible, it does illustrate an example for how uncertainty is typically intended to capture unknowns such as neurodevelopmental hazards. In this risk framework, SAT has identified the following UF as appropriate to consider either individually or combined:

- UF = 10 for estimating a no effect level from an effect level.
- UF = 3 or 10 for human variability in susceptibility which addresses variations among the susceptible population.
- UF = 3 or 10 for database uncertainty which addresses limitations in the available evidence and future unknowns.

As an example, we can take a drinking water fluoride concentration of 4 mg/L, which was the highest effect level observed between fluoride drinking water concentrations and neurodevelopment (Taylor et al 2025), apply a single UF = 10, and arrive at 0.4 mg/L fluoride in drinking water as an example protective value against neurodevelopmental effects.

Considerations

Based on the risk framework above and recognizing that the risks detailed above are associated with ingestion of fluoride; SAT provides the following considerations to carry forward to reduce the risk of neurodevelopmental impacts of fluoride:

- The consistency of the findings for effects on IQ across a large body of evidence, the proximity between the current recommended benefit levels (0.7 mg/L, U.S. Health and Human Services 2015) and the new hazard levels, and the seriousness of IQ decrements warrant careful consideration of this new risk in public health decision-making. Future risk/benefit analyses on fluoride should carefully weigh neurodevelopmental hazards alongside the oral health benefit attributed to the intervention when establishing appropriate exposure limits for fluoride in drinking water and other sources.
- It is best practice to incorporate uncertainty in risk. While the oral health benefits of fluoride may make the selection of an uncertainty factor of one defensible (i.e. UF = 1), it imparts no buffer to account for variability and future unknowns.
- It is not within the scope of this risk framework to discuss the benefits of water fluoridation. However, this is an important decision point that requires a clear and robust understanding of the benefits, especially as the benefit is primarily for the susceptible population at risk of neurodevelopmental effects (e.g. developing fetus, infants, and children).
- An additional burden for pregnant people and caregivers may occur based on fluoridation status if recommendations are made to purchase and use alternate water during pregnancy and for mixing formula.
- Should a source of fluoride be removed from the population it is possible an increase in caries may occur, particularly in children. It will be important for communities to consider how to improve access to oral health care.
- The science surrounding fluoride and toxicity continues to grow and should be monitored. As our ability to measure endpoints with greater sensitivity improves, so does our ability to learn more about and identify additional modes of toxicity. It is likely in the future that existing modes of toxicity (skeletal and dental fluorosis and neurodevelopmental effects) are further defined and that new modes may be better identified (e.g. endocrine disruption via thyroid) through research.
- The federal landscape surrounding fluoride appears to be shifting and changes in guidance could occur that may have implications for the way fluoride is used in oral health.

Potential Recommendations

For Pregnant Individuals

- Minimize fluoride ingestion to protect fetal neurodevelopment. This includes avoiding swallowing fluoridated toothpaste during brushing and limiting consumption of foods and beverages high in fluoride content.
- Consider using alternative water sources for drinking and cooking if living in areas with naturally high fluoride levels or fluoridated water.
- Continue to maintain good oral hygiene through proper brushing techniques and regular dental check-ups throughout pregnancy.

For Formula-Fed Infants

- Avoid using fluoridated water for reconstituting infant formula. It is worth noting that breast milk naturally contains minimal fluoride.
- Caregivers might consider ready-to-feed formula options that do not require reconstitution.
- Parents should consult with pediatricians and pediatric dentists about appropriate fluoride exposure for infants.

For Young Children

- Parents should consult pediatricians and pediatric dentists about appropriate usage of fluoridated products and supplements.
- Parents should supervise young children during brushing to ensure they do not swallow toothpaste.

For Older Children and Adults

- When using fluoridated products, follow all instructions on product labels. Do not swallow fluoridated products.
- Consult with their doctor and/or dentist for any specific concerns.

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Attachment B: Summary of Responses of Other States and Organizations

There are 14 states that require fluoridation in some form:

Arkansas, California, Connecticut, Delaware, Georgia, Illinois, Kentucky, Louisiana, Minnesota, Mississippi, Nebraska, Nevada, Ohio, and South Dakota.

Nevada state law only requires water suppliers to add fluoride if they serve more than 100,000 people in a county with a population of more than 700,000. This criteria includes only Clark County.

Almost all state requirements include exceptions. The most common are for water suppliers serving less than a designated amount of people, like 10,000 in California or 500 in South Dakota.

Other exceptions include allowing local jurisdictions to vote not to add fluoride, like in Georgia or Nebraska, or making it contingent on state funding as in Mississippi or Louisiana. In Louisiana's case, state funding has not been provided since federal grant funding ended in 2015.

As of August 2025, 2 states have banned fluoridation:

- Utah became the first state to ban water fluoridation with HB 81, which prohibits the addition of fluoride to a public water system. It took effect May 7, 2025.
- Florida's SB 700 took effect on July 1, 2025, and prevents local governments from using any "water quality additive", including fluoride, that is not used to improve water quality or remove contaminants.

Some other common state requirements around fluoride and water fluoridation include:

- Setting fluoride concentrations for water suppliers who do add fluoride (typically at or around 0.7 mg/L).
- Requiring water suppliers to conduct regular fluoride concentration tests (ranging from daily to monthly).
- Requiring water suppliers who cease or begin adding fluoride, or who exceed a certain fluoride concentration limit, to issue public notices.
- Placing limits on what state entities are allowed to enforce.
    - For instance, Michigan state entities cannot require fluoridation, and Missouri's Dept. of Natural Resources cannot require or prohibit fluoridation.
- Requiring jurisdictions to hold votes on fluoridation, as in New Hampshire.
- Setting up fluoridation grant programs to support water suppliers' fluoridation efforts, such as in New York or South Carolina.

Many bills have been introduced around fluoridation in recent years. This includes at least 2 states, Hawaii and New Jersey, which introduced bills to require fluoridation.

At least 16 states have introduced bills to ban fluoridation similar to Utah and Florida: Alaska, Arkansas, Hawaii, Louisiana, Maine, Massachusetts, Minnesota, Montana, New Hampshire, North Carolina, North Dakota, Ohio, Pennsylvania, South Carolina, Tennessee, and Texas.

In Texas, the Agriculture Commissioner has recently called on the governor and legislature to ban fluoridation.

Lastly, at least 3 states with fluoride requirements saw bills introduced to make fluoridation optional: Kentucky, Nebraska, and South Dakota.

In addition to the recent bans in Utah and Florida, some states have updated rules around fluoridation in recent years.

In Iowa, the Public Health Department rescinded its community water fluoridation grant program in May 2025 due to its being unfunded by the federal government for several years.

In Nebraska, the Department of Health and Human Services adjusted the required fluoride concentration in its rules, lowering the optimal range of 1 to 1.3 parts per million to a concentration of 0.7 mg/L.

In Virginia, Board of Health rules previously allowed the Board to *require* fluoridation where practicable and feasible, but in 2021 this was amended to a recommendation.

At least 8 states have released some sort of publication in the past 5 years reaffirming support for fluoridation: Alabama, Colorado, Hawaii, Maine, New Hampshire, New York, Oklahoma, and Vermont. 2 others, Ohio and North Carolina, are in the process of reviewing documents related to fluoridation.

At least 2 states, Texas and Florida, have seen state officials come out against fluoridation in recent years. Florida's Surgeon General issued guidance in 2024 against fluoridation, citing a number of studies which have since been criticized by experts. Texas' Agriculture Commissioner issued a statement calling on the governor and legislature to ban fluoridation. The statement voiced support for HHS Secretary Robert Kennedy's anti-fluoridation stance but did not cite specific studies.

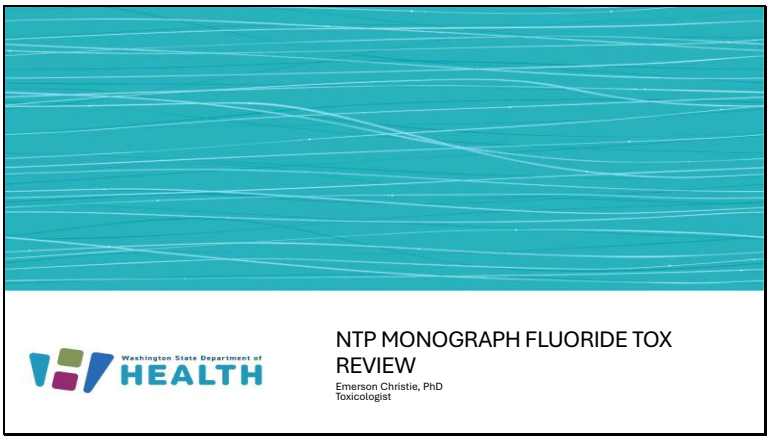
As of August 2025, we are not aware of any state or national organization that has changed its fluoride policy or recommendation as a result of updated science regarding potential harms of fluoride.

Attachment C: Information presented in the panel sessions

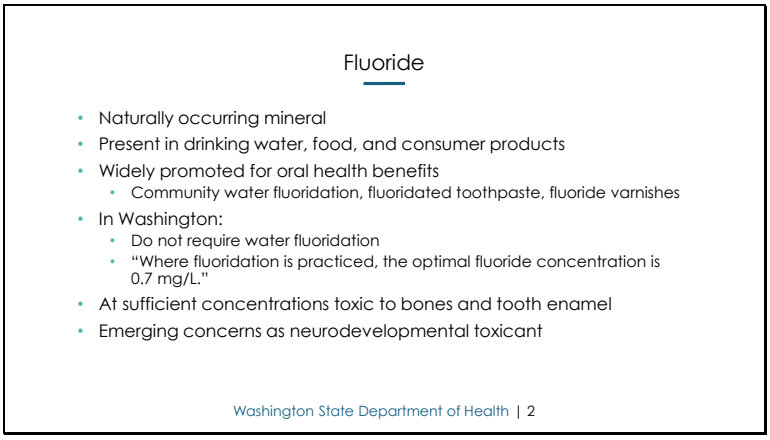
Slides, as available, from presentations

January 9; Emerson Christie; Review of the NTP Monograph

Slide 1



Slide 2



Slide 3

National Toxicology Program

- National Toxicology Program (NTP)
  - Federal Toxicology Program - interagency program within the U.S. Department of Health and Human Services
  - Tasked with identifying compounds with toxic and biological effects and providing information for public health decisions
  - Performed studies on many contaminants including lead and PFAS
  - NTP PFAS study serves as the basis for WA SAL for PFHxS

Slide 4

The NTP Monograph: History

- 2006 National Research Council
  - Association between high levels of fluoride in drinking water and adverse neurological effects in humans
- 2016 NTP Review of Animal Studies
  - Low to moderate confidence fluoride impacts learning and memory
- August 2024 Fluoride Monograph:
  - State of the science – Review
  - Moderate confidence, that higher estimated fluoride exposures (e.g., >1.5 mg/L in drinking water) are consistently associated with lower IQ in children

Slide 5

What this report does not say

- It does not address whether the sole exposure to fluoride at 0.7 mg/L is associated with a measurable effect on IQ
- The monograph does not advocate for discontinuing CWF
- It does not provide a risk assessment
- It does not provide a oral health benefit assessment
- It does not provide a risk/benefit analysis

This is not bad news – this is good news

Slide 6

OHAT Approach

Systematic Review
- Planning and protocol development
- Identify evidence
  - Comprehensive literature search
  - Literature screening
- Evaluate evidence
  - Extract data
  - Risk of bias assessment

Evidence Integration
- Rate confidence in association between exposure and outcome

Slide 7

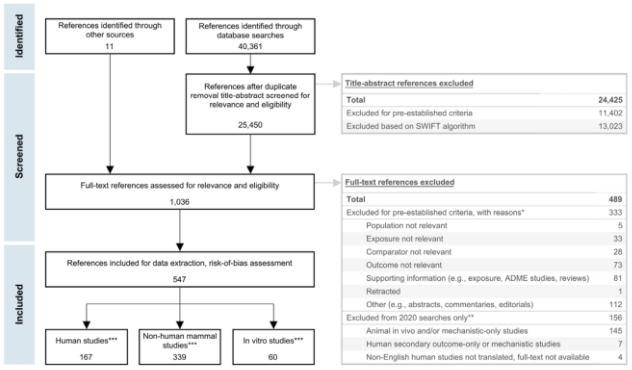


Figure 2. Study Selection Diagram[a]

Figure credit: NTP 2024

Slide 8

What makes a study low (or high) risk of bias

- OHAT approach, some risk-of-bias questions or elements are considered potentially more important when assessing studies
- Key questions
  - Confounding
    - Required - age, sex, and socioeconomic status
    - Required where applicable - co-exposures (lead and arsenic), other covariates considered important for population and outcome
    - Not required to address every covariate
  - Exposure characterization
    - Assessed in a variety of ways – satisfied as long as efforts to reduce bias were included
  - Outcome assessment
    - IQ –appropriate for population and standardized, assessors blind

Slide 9

The NTP Monograph: IQ Association

Monograph (1989 – May 2020)

- 19 low risk of bias studies
  - None from the U.S. (2 Canada)
  - 18 showed inverse association
  - Overall 95% inverse
- 53 medium to high risk of bias studies
  - 46 showed inverse association
  - Overall 86% inverse

Addendum (May 2020 – Oct 2023)

- 12 additional low risk of bias studies
  - 12 inverse associations
  - Overall 97% inverse
- 16 additional medium to high risk of bias studies
  - 13 showed inverse association
  - Overall 86% inverse

Slide 10

The NTP Monograph: IQ Association

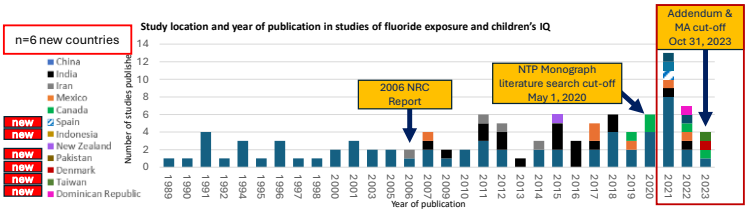


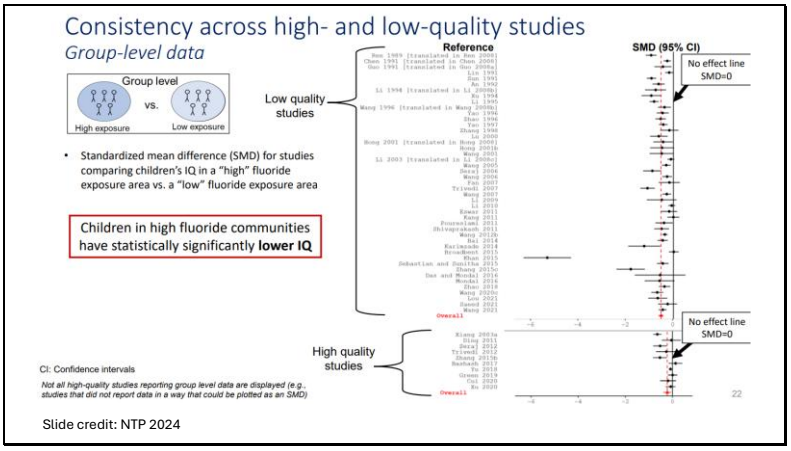
Figure credit: NTP 2024

Slide 11

NOTE

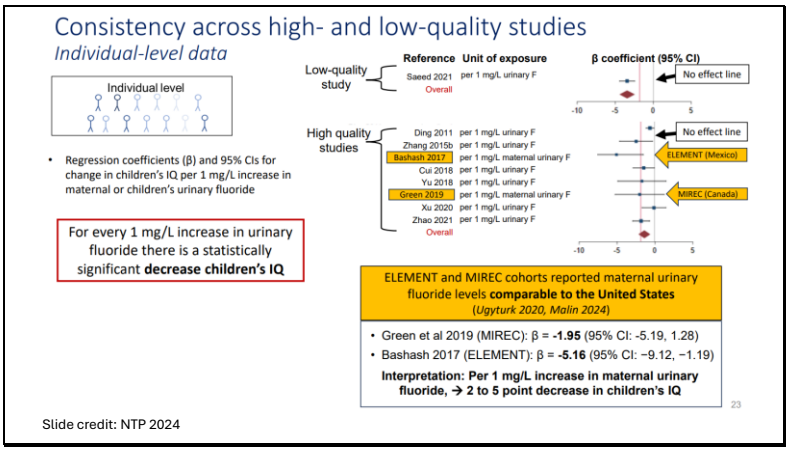
- The next 4 slides are from the NTP slide deck: Fluoride, Neurodevelopment, and Cognition: A National Toxicology Program Monograph. https://www.healthandenvironment.org/assets/images/taylor-slides-dec-3-2024.pdf
- This presentation was put on by the Collaborative for Health and Environment on 3 December 2024.
- Slides have been stripped of WA DOH branding and attribution is on each slide.

Slide 12



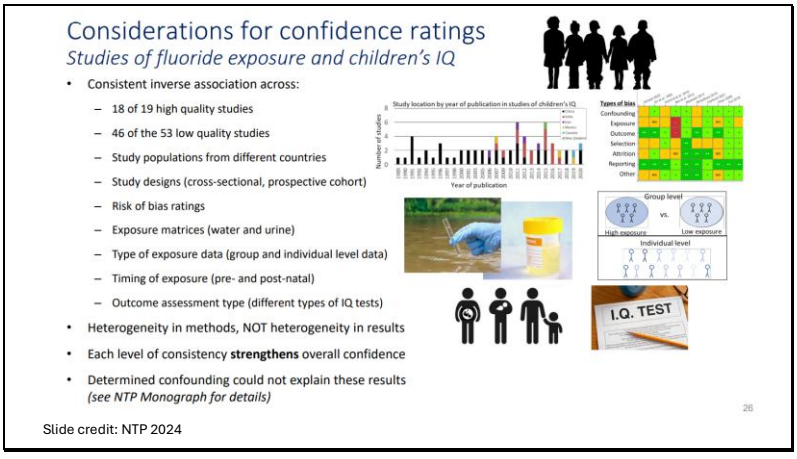
Slide credit: NTP 2024

Slide 13



Slide credit: NTP 2024

Slide 14



Slide credit: NTP 2024

Slide 15

Of note…



- Final confidence conclusions based primarily on high-quality studies (i.e., the best evidence)
  - Consideration of low-quality studies does not decrease confidence in overall body of evidence
- Conclusions based primarily on non-US studies where total fluoride exposure approximated *>1.5 mg/L fluoride in drinking water
  - Several high-quality prospective birth cohort studies with maternal urinary fluoride levels comparable to the United States

  >1.5 mg/L refers to WHO Drinking Water Guideline of 1.5 mg/L; chosen to describe "higher" fluoride exposure in the NTP Monograph based on an overall assessment of the epidemiology literature; represents a useful total fluoride exposure equivalent metric (no alternative safety guidelines for total fluoride exist)
- Review ***does not***
  - Evaluate benefits of fluoride or provide a risk/benefit analysis
  - Address whether ***sole exposure*** to fluoride at 0.7 mg/L in drinking water is associated with neurodevelopment and cognitive effects
- Targeted research that prospectively examines the association between fluoride exposure and children's IQ in optimally fluoridated areas of the United States would add clarity to the existing data at lower levels

29

Slide credit: NTP 2024

Slide 16

Meta Analyses Summary

- All meta analyses in the NTP report showed an inverse association between fluoride and IQ
- Kumar et al 2023 – Split studies into high and low fluoride exposure – high showed an inverse, low did not

Addendum Table 2. Previous Meta-analyses on Exposures to Fluoride and Children's IQ

Analysis	Number of Studies	Pooled Effect Type, Estimate (95% CI)	Heterogeneity	
			p-value	I^2
Tang et al. (2008)	16	WMD, −5.03 (−6.51, −3.55)	NR	NR
Choi et al. (2012)	27	SMD, −0.45 (−0.56, −0.34)	<0.001	80%
Duan et al. (2018)	26	SMD, −0.52 (−0.62, −0.42)	<0.001	69.1%
Miranda et al. (2021)	10	OR, 3.88 (2.41, 6.23)	<0.0001	77%
Veneri et al. (2023)	30 (38 results)	WMD, −4.68 (−6.45, −2.92)	NR	98.75%
Kumar et al. (2023)	28 (31 results)	SMD, −0.33 (−0.44, −0.22)	<0.001	83%
DTT Meta-analysis, Taylor et al. (2024, in press)[a]	59	SMD, −0.45 (−0.57, −0.33)	<0.001	94%

CI = confidence interval; NR = not reported; SMD = standardized weighted mean difference; OR = odds ratio of low IQ in the high fluoride versus low fluoride groups; WMD = weighted mean difference.
[a]The NTP authors of this monograph conducted a companion systematic review and meta-analysis of fluoride exposure and children's IQ (DTT Meta-analysis, Taylor et al. 2024, in press).

Slide 17

Is maternal urinary fluoride an acceptable measurement?

- Maternal Urinary Fluoride is acceptable
  - Measures total fluoride exposure – desired from a risk and hazard perspective
  - Practicality – easy to obtain
  - Temporality
    - Pro – measures during specific time periods
    - Con – subject to bias but can be controlled

Slide 18

Why IQ?

- Measures population-based shifts in various cognitive parameters
- Important toxicological measurement
  - Lead, arsenic, mercury, and PCBs are neurotoxicants as measured by IQ
  - Exposure relationship often dose-dependent and well-characterized
- Shifts in population IQ associated with societal impacts
  - Educational outcomes
  - Health outcomes
  - Income (when controlling for socioeconomic factors)
  - Crime

Slide 19

What is the magnitude of impact?

- For every 1 mg/L in increased maternal urinary fluoride (MUF) => 1.14 – 1.63 IQ points (Taylor et. al. 2025)
- MUF third trimester Los Angeles CA cohort median 0.8 mg/L and 95 C.I. of 1.89 mg/L (Malin et. al. 2023)
- ~1 IQ point at median and 2-3 IQ points at 95th C.I. (typical protection level)
- For context - estimated average impact of lead is 2.6 IQ points (McFarland 2022)
- Any contributing shift leftward from a population perspective is of note

Slide 20

What is the window of exposure?

- Most studies are childhood
  - Of 19 low risk of bias studies
    - Three with maternal urine
    - One breastfeeding and formula
    - Others are either urine or drinking water

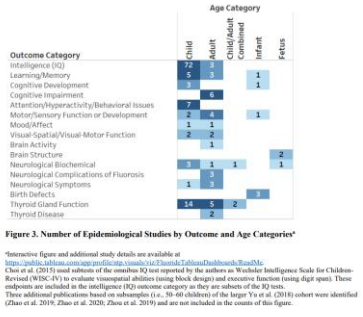


Figure 3. Number of Epidemiological Studies by Outcome and Age Categories[a]

[a]Interactive figure and additional study details are available at https://public.tableau.com/app/profile/ntp.visuals/viz/TheirdcTableauDashboards/ReadMe.
Choi et al. (2015) used subtests of the omnibus IQ test reported by the authors as Wechsler Intelligence Scale for Children-Revised (WISC-IV) to evaluate visuospatial abilities (using block design) and executive function (using digit span). These endpoints are included in the intelligence (IQ) outcome category as they are subsets of the IQ tests.
Three additional publications based on subsamples (i.e., 50–60 children) of the larger Yu et al. (2018) cohort were identified (Zhao et al. 2019; Zhao et al. 2020; Zhou et al. 2019) and are not included in the counts of this figure.

Slide 21

Why might this matter for CWF?

- Risk
  - There must exist a margin between the exposure and the level at which a hazard is present.
- Reality

Total fluoride = 1.5 mg/$L_{drinking\ water}$ + $0_{other\ sources}$ => expect lower IQ

However, reality is

Total fluoride = 0.7 mg/ $L_{drinking\ water}$ + $X_{other\ sources}$ => ?

Slide 22

Why might this matter for CWF? Risk

- Risk
  - Conventional risk assessment methods

$$Safe\ level = \frac{LOAEL/NOAEL}{[UF]}$$

  - Fluoride NTP = 1.5 mg/L (LOAEL)
  - Uncertainty Factors (UF) => 10 for LOAEL to NOAEL and 3 for intraspecies

$$Safe\ level = \frac{1.5\ mg/L}{10 * 3} = 0.05\ mg/L$$

  - Fluoride MCL = 4 mg/L (LOAEL)

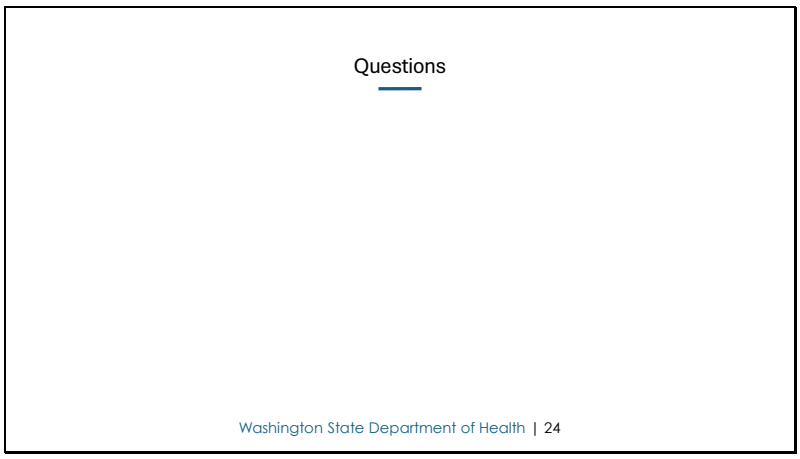
$$Safe\ level = \frac{4\ mg/L}{10 * 3} = 0.13\ mg/L$$

Slide 23

Why might this matter for CWF? Reality

- Reality
  - 0.7 mg/ $L_{drinking\ water}$ + $X_{other\ sources}$ => ?
  - Many sources of fluoride including fluoridated products
  - Potential for exposure at a hazardous level
- Recent data indicate potential risk at current exposures:
  - For every 1 mg/L in increased MUF => 1.14 – 1.63 IQ points (Taylor et. al. 2025)
  - MUF third trimester Los Angeles CA cohort median 0.8 mg/L and 95 C.I. of 1.89 mg/L (Malin et. al. 2023)
  - ~1 IQ point at median and 2-3 IQ points at 95th C.I. (typical protection level)
  - Where water fluoridation occurs ~50% of maternal urinary fluoride (MUF) is attributable to water consumption (Till et. al. 2018)

Slide 24



Slide 1



TOXIC SUBSTANCES CONTROL ACT (TSCA)

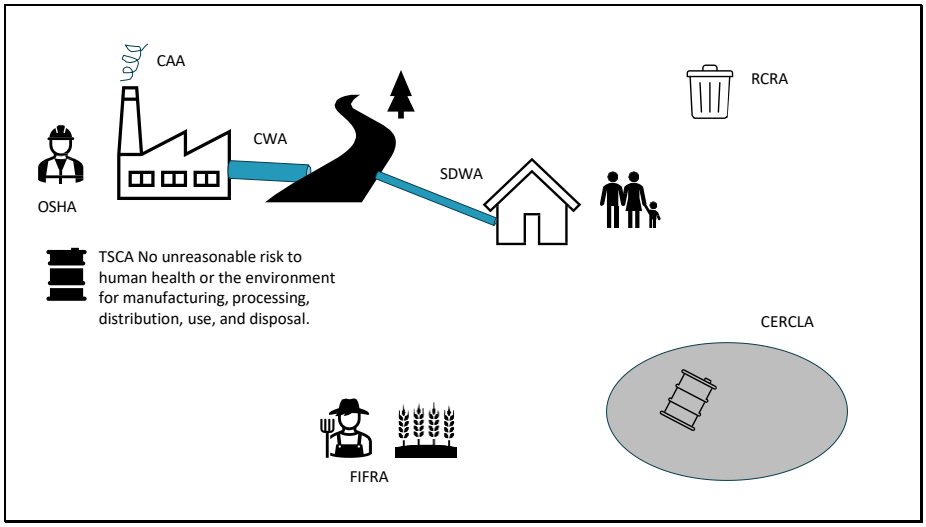
Holly Davies, PhD
Environmental Public Health
Fluoride Science Review
Jan. 28, 2025

Slide 2

Toxics Substances Control Act (1976)

- Major federal law that regulates toxic chemical manufacturing, processing, distribution, use, and disposal.
- No unreasonable risk for the intended or foreseen uses.
- Certain substances are generally excluded from TSCA (Sec. 3(2)(B)), including tobacco, pesticide, food, drugs, cosmetics
  - any article subject to the tax imposed by section 4181 of the Internal Revenue Code of 1954
- There are other federal laws within EPA to protect human health and the environment

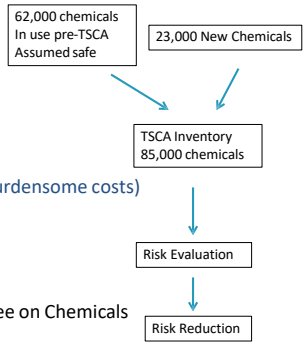
Slide 3



Slide 4

TSCA selected sections with 2016 changes

- Sec. 4 Testing
  - Easier to require testing by order
  - Direction to phase out the use of animals in testing
- Sec. 5 New Chemical review
  - Deadlines for EPA review
- Sec. 6 Management of existing chemicals, ban on PCBs
  - Risk evaluation without consideration of costs (not least burdensome costs)
- Sec. 8 Inventory, reporting, and record keeping
- Sec. 18 Preemption- almost entirely changed in 2016
- Sec. 21 Citizens petitions under sections 4, 5, 6 or 8.
- Sec. 26 Administration, evidence, Science Advisory Committee on Chemicals



Slide 5

Sec. 6 Prioritization, Risk Evaluation, and Regulation of Chemical Substances and Mixtures

(a) If the Administrator determines that the **manufacture, processing, distribution, use, or disposal** presents an **unreasonable risk**, they **shall by rule** remove such risk.
Prohibit, limit use, warning label, notifications, record keeping

(b) Risk Evaluations- Prioritization, requirements of the evaluations
Not consider costs or other nonrisk factors
Deadlines

(c) Rulemaking
Shall publish … a final rule … **not later than 2 years after**
Consider costs and benefits, feasible alternatives

(g) Exceptions for specific conditions of use, time limited
Essential with no alternative, **significantly disrupt** the economy, etc.
provides a substantial benefit to health, the environment, or public safety

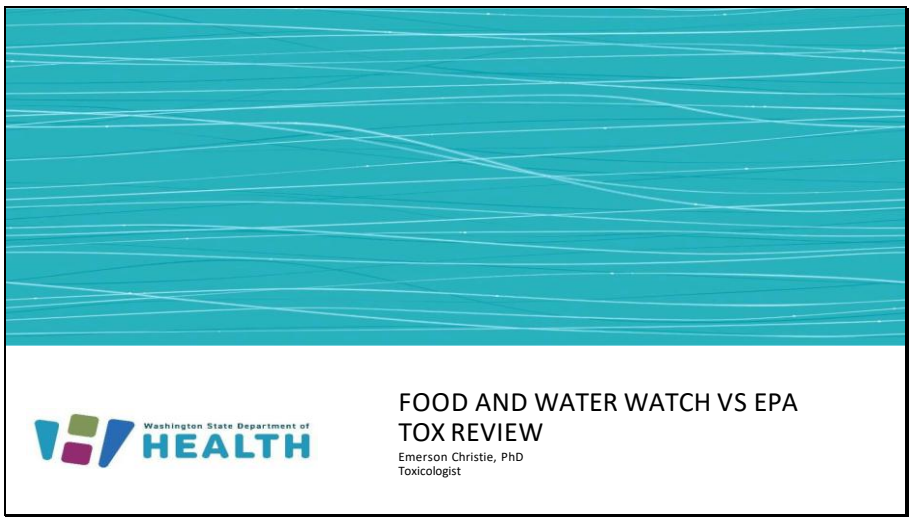
(i) Final Agency Actions and state preemption (Sec. 18)
No unreasonable risk (b) means states are preempted
Unreasonable risk (b) and final rule (a) states are preempted

Slide 6

Fluoride Sec. 21 Petition

- 2016 A group of NGOs petitioned EPA under Sec.21 to ban fluoridation of drinking water under Sec. 6
- 2017 EPA denied the petition saying the petition had to include a risk evaluation as in Sec. 6(b) and weight of scientific evidence as in Sec. 26
- NGOs appealed under Sec. 21 to a federal district court and bench trial in 2020 and 2024
- 2024 Judged ordered EPA must take actions under Sec. 6 to eliminate the unreasonable risk

Slide 1



Slide 2

Issue

5 The issue before this Court is whether the Plaintiffs have established by a preponderance
6 of the evidence that the fluoridation of drinking water at levels typical in the United States poses
7 an unreasonable risk of injury to health of the public within the meaning of Amended TSCA. For

Slide 3

Risk Assessment & Determination

- Hazard Assessment
  - Hazard identification, weight of evidence
  - Dose response analysis – point of departure (POD)
- Exposure Assessment
  - Level of exposure
  - Populations
- Risk Characterization
  - Uncertainty
  - Margin of exposure

Risk Assessment

- Risk Determination
  - Summary of assessment
  - Identification of "unreasonable risk"

Slide 4

Hazard Assessment

- Hazard Identification
  - Causation **not** a requirement to establish neurotoxicity hazard
- Weight of the evidence
  - Directional and consistent
- Dose response assessment and point of departure
  - Benchmark

Slide 5

Hazard Assessment

- Hazard Identification:
  - Causation **not** a requirement to establish neurotoxicity hazard

40. EPA experts agreed, in line with the NTP Monograph's conclusion, that fluoride is associated with adverse IQ in children at "higher" levels of exposure. Namely, Dr. Barone testified that he agreed that there is "something going on" at higher-dose levels, though unclear about where the threshold is. Dkt. No. 415, Feb. 12, 2024, Trial Tr. at 1372:9-1373:9 (Barone). Dr. Barone agreed that, at 4 mg/L of fluoride exposure and above, there is more data to support a finding of an adverse effect associated with fluoride. *Id.* at 1373:1-9 (Barone). Dr. Barone further

Slide 6

Hazard Assessment

✓ Hazard Identification:
  - Causation **not** a requirement to establish neurotoxicity hazard
- Weight of the evidence
  - Directional and consistent

EPA's expert, Dr. Barone agreed that the NTP Monograph is a "high quality review." Dkt. No. 440, Feb. 13, 2024, Trial Tr. at 1427:2-4 (Barone). Accordingly, the Court finds that the NTP Monograph is probative and afforded significant weight in the risk evaluation analysis.

55. In conclusion, this evidence is sufficient to proceed to the dose-response assessment of the analysis. *Cf.* Methylene Risk Evaluation at 262 (conducting dose-response analysis for Methylene under Amended TSCA based upon one animal study).

Slide 7

Hazard Assessment

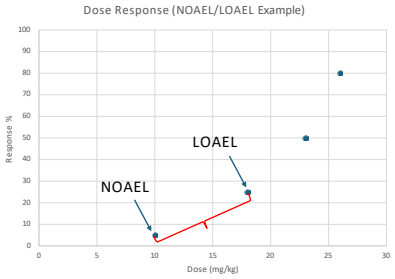
- ✓ Hazard Identification:
  - Causation **not** a requirement to establish neurotoxicity hazard
- ✓ Weight of the evidence
  - Directional and consistent
- Dose response assessment and point of departure

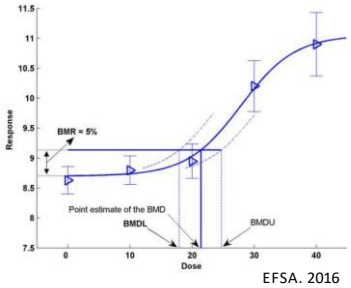
60. 0.28 mg/L, or alternatively, 0.768 and/or 1.536 mg/L measured in maternal urinary fluoride is associated with a 1-point decrease in IQ of girls and boys and is a legitimate point of departure (BMCL) to use in this risk evaluation.

61. Alternatively, 4 mg/L measured in either urinary fluoride or water fluoride, is a legitimate, conservative point of departure (LOAEL) to use in the risk evaluation.

Slide 8

Hazard Assessment: Point of Departure (POD)





EFSA. 2016

Slide 9

Hazard Assessment: Benchmark Dose

- Where did these numbers come from?

60. 0.28 mg/L, or alternatively, 0.768 and/or 1.536 mg/L measured in maternal urinary fluoride is associated with a 1-point decrease in IQ of girls and boys and is a legitimate point of departure (BMCL) to use in this risk evaluation.

61. Alternatively, 4 mg/L measured in either urinary fluoride or water fluoride, is a legitimate, conservative point of departure (LOAEL) to use in the risk evaluation.

Slide 10

Hazard Assessment: Benchmark Dose

- Court considered two publications
  - Phillipe Grandjean (2022 & 2023)
  - Include "lower" exposure levels measured in maternal urinary fluoride
    - 0.9 mg/L in ELEMENT (Mexico)
    - 0.42 mg/L in MIREC (Canada)
    - 0.58 mg/L in OCC (Denmark)
  - These values are consistent with an LA cohort
  - Dr. Grandjean well respected in environmental neurotoxicants and benchmark dose modeling
    - Literal EPA textbook example
    - EPA has used Grandjean BMDs in other decisions

Slide 11

Hazard Assessment: Benchmark Dose

- Benchmark dose analysis to determine level of maternal urinary fluoride (MUF) associated with 1 point drop in IQ in offspring
- Grandjean 2023
  - ELEMENT, MIREC, and OCC (Canada, Mexico, and Denmark)
  - BMCL (Benchmark Concentration Lower Bound) = **0.28 mg/L MUF**
  - Did not publish a squared model
- Grandjean 2022
  - ELEMENT and MIREC (Canada and Mexico)
  - BMCL = 0.20 mg/L MUF
  - Linear model identified as best overall; split linear and a squared model
  - Squared model: BMCL = **0.768 mg/L MUF**
  - With an uncertainty factor of 2 for not having the OCC data: BMCL = **1.536 mg/L**

Slide 12

Hazard Assessment: LOAEL

12 establishes with consistency an association with reduced IQ at that level. Specifically, the NTP
13 Meta-analysis observed a statistically significant inverse association between fluoride and reduced
14 IQ at 4 mg/L measured in water fluoride, based on low-risk-of-bias/high quality studies (*i.e.*, 6

- 4 mg/L in drinking water is also the MCL for skeletal fluorosis
- 2 mg/L in drinking water secondary (non-enforceable) MCL dental fluorosis

Slide 13

Hazard Assessment: LOAEL

Dr. Barone agreed that, at 4 mg/L of fluoride exposure and above, there is more data to support a finding of an adverse effect associated with fluoride. *Id.* at 1373:1-9 (Barone). Dr. Barone further

81. The EPA has identified a LOAEL based upon far less evidence than that in the record before this Court. In the EPA's risk evaluation of Methylene, conducted pursuant to Amended TSCA, it used a LOAEL for developmental neurotoxicity, derived from the analysis of ***one study conducted upon mouse pups*** (Fredriksson et al., 1992). *See* Methylene Risk Evaluation at 262.

Slide 14

Hazard Assessment

✓ Hazard Identification:
- Causation **not** a requirement to establish neurotoxicity hazard

✓ Weight of the evidence
- Directional and consistent

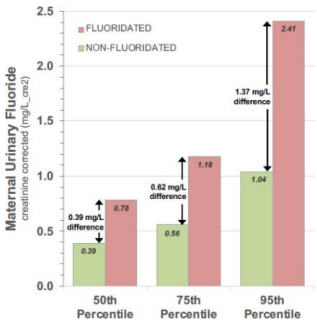
✓ Dose response assessment and point of departure

Fluoride is a neurotoxicity hazard

Slide 15

Exposure Assessment

- What is the exposure level at the condition of use?
  - Till et al 2018 – 0.56 mg/L water fluoride
  - Malin et al 2023 – community water fluoridation Los Angeles (~0.7 mg/L)
- Third trimester (Malin et al 2023)
  - Median MUF = 0.8 mg/L
  - 95 CI MUF = 1.89 mg/L
- ~50% MUF attributed to drinking water
- Default RSC for contaminant in drinking water would be 20%



Till et al 2018

Slide 16

Exposure Assessment: Maternal Urinary Fluoride

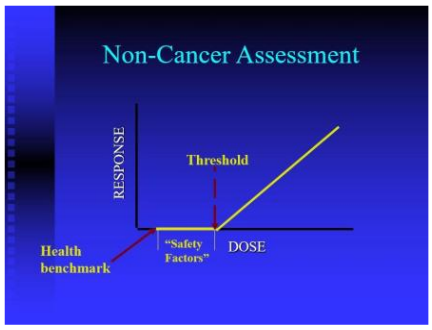
- Maternal Urinary Fluoride is acceptable
  - Measures total fluoride exposure
    - Desired from a risk and hazard perspective
    - TSCA specifically allows for consideration of aggregate exposures
  - Water fluoride concentrations:
    - Consistently associated with urinary fluoride
    - Represent ~50% of observed maternal urinary fluoride
  - Temporality
    - Measures during specific time periods

And the EPA's expert witness agreed that the increase in maternal urinary fluoride levels can largely be attributed to intake of fluoridated water. Dkt. No. 416, Feb. 13, 2024, Trial Tr. at

Slide 17

Risk Characterization

- Point of departure (i.e. hazard level) compared to exposure level
- Point of departure is inadequate for protection – a margin must exist



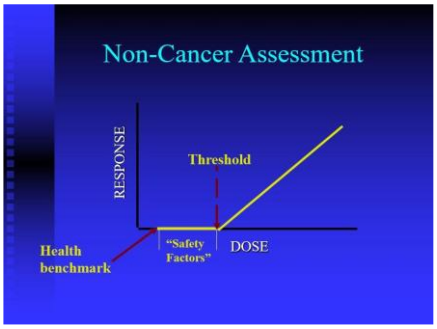
O'Garro 2025

Slide 18

Risk Characterization: Uncertainty Factors

- Intraspecies variability
- Interspecies variability
- LOAEL to NOAEL
- Database uncertainty
- Subchronic to chronic

- Typically single order of magnitude (i.e. 10 fold)
  - Can be ½ power (i.e. 3 fold)
- Total UF should not exceed 3000



O'Garro 2025

Slide 19

Risk Characterization: Conventional Methods

- Conventional risk assessment methods

$$Safe\ level = \frac{POD}{[UF]}$$

  - POD = Point of departure => BMCL, NOAEL, LOAEL
  - UF = Uncertainty Factors
- Determined risk was present at all identified PODs:
  - BMCL = 0.28 mg/L MUF
  - BMCL = 0.768 mg/L MUF
  - BMCL = 1.536 mg/L MUF
  - LOAEL = 4 mg/L water fluoride

Slide 20

Risk Characterization: Conventional Methods

- Conventional risk assessment methods
  - POD = 0.28 mg/L MUF (Grandjean 2023 – linear model)
  - UF = 10 for intraspecies variability

$$Safe\ level = \frac{0.28\ mg/L}{10} = 0.028\ mg/L$$

- 0.028 mg/L is:
  - Less than the median MUF of 0.8 mg/L
  - Less than the 95 CI MUF of 1.89 mg/L
  - Less than the median MUF_{water} of 0.4 mg/L (i.e. 50% of the total MUF)
  - Less than the 95 CI MUF_{water} of 0.945 mg/L (i.e. 50% of the total MUF)

  - Risk is present

Slide 21

Risk Characterization: Conventional Methods

- Conventional risk assessment methods
  - POD = 0.768 mg/L MUF (Grandjean 2022 – squared model)
  - UF = 10 for intraspecies variability

$$Safe\ level = \frac{0.768\ mg/L}{10} = 0.0768\ mg/L$$

- 0.0768 mg/L is:
  - Less than the median MUF of 0.8 mg/L
  - Less than the 95 CI MUF of 1.89 mg/L
  - Less than the median MUF_{water} of 0.4 mg/L (i.e. 50% of the total MUF)
  - Less than the 95 CI MUF_{water} of 0.945 mg/L (i.e. 50% of the total MUF)

  - Risk is present

Slide 22

Risk Characterization: Conventional Methods

- Conventional risk assessment methods
  - POD = 1.536 mg/L MUF (Grandjean 2022 – squared model x2 for uncertainty)
  - UF = 10 for intraspecies variability

$$Safe\ level = \frac{1.536\ mg/L}{10} = 0.1536\ mg/L$$

- 0.1536 mg/L is:
  - Less than the median MUF of 0.8 mg/L
  - Less than the 95 CI MUF of 1.89 mg/L
  - Less than the median MUF_{water} of 0.4 mg/L (i.e. 50% of the total MUF)
  - Less than the 95 CI MUF_{water} of 0.945 mg/L (i.e. 50% of the total MUF)
  - Risk is present

Slide 23

Risk Characterization: Conventional Methods

- Conventional risk assessment methods
  - POD = 4 mg/L water fluoride (NTP meta analysis)
  - UF = 10 for intraspecies variability
  - UF = 10 for LOAEL to NOAEL

$$Safe\ level = \frac{4\ mg/L}{10\ x\ 10} = 0.04\ mg/L$$

- 0.04 mg/L is:
  - Less than the community water fluoridation level of 0.7 mg/L
  - Risk is present

Slide 24

Risk Determination

- "Unreasonable" risk
  - Severity of the hazard
  - Exposure-related considerations
    - Duration
    - Magnitude
    - Population size
  - Population characteristics
  - Confidence in the information used for hazard and exposure
  - Confidence in uncertainties and assumptions

Slide 25

Risk Determination

- "Unreasonable" risk
  - Severity of the hazard
    - IQ loss
  - Exposure-related considerations
    - 2,000,000 pregnant people
    - over 300,000 exclusively formula-fed babies
  - Population characteristics
    - Very susceptible populations - pregnant people and infants
  - Confidence in the information used for hazard and exposure
    - High level of certainty of hazard between fluoride and IQ
    - Some uncertainty in which POD to use
  - Confidence in uncertainties and assumptions
    - Uncertainty in mechanism of action

Slide 26

Finding

IV. CONCLUSIONS OF LAW

121. Plaintiffs have proven, by a preponderance of the evidence, that water fluoridation at the level of 0.7 mg/L – the prescribed optimal level of fluoridation in the United States – presents an "unreasonable risk of injury to health or the environment, without consideration of costs or other non-risk factors, including an unreasonable risk to a potentially exposed or susceptible subpopulation under the conditions of use." 15 U.S.C. § 2620(b)(4)(B)(ii).

122. The Court thus orders the Administrator to initiate rulemaking pursuant to Subsection 6(a) of TSCA. *See id.* §§ 2605(a), 2620(a).

Slide 27

Final Thoughts on Risk

- Similar assessments could be done with other PODs for fluoride
- This assessment did not consider benefits
- Did not identify what action may be taken

This is not bad news – this is good news

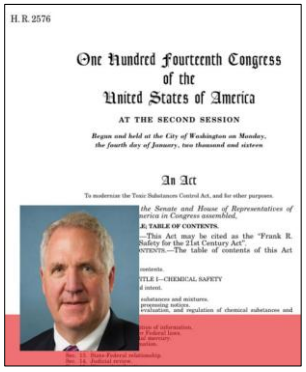
Slide 1

Food & Water Watch, Inc.
v.
U.S. Environmental Protection Agency (EPA)

US District Court Northern District of California
Case No. 17-cv-02162-EMC

Slide 2

Background


H. R. 2576

One Hundred Fourteenth Congress
of the
United States of America

AT THE SECOND SESSION

Begun and held at the City of Washington on Monday, the fourth day of January, two thousand and sixteen

An Act

To modernize the Toxic Substances Control Act, and for other purposes.



Washington State Department of Health | 2

Slide 3

Procedural History

- June 2016, US Congress passes law to amend TSCA
- November 2016, citizens petition, including Food & Water Watch, to EPA to regulate fluoridation
- February 2017, EPA denied Plaintiffs
- April 2017, Plaintiff filed suit in 9th Circuit

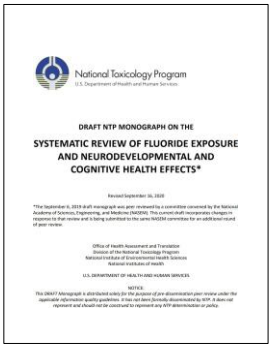


Washington State Department of Health | 3

Slide 4

Trial Phase 1

- June 2020, 7-day bench trial
- August 2020, Court stayed to allow EPA to consider NTP Systematic Review
- Plaintiffs filed supplemental petition for EPA TSCA reconsideration
- EPA denied petition


National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONOGRAPH ON THE
SYSTEMATIC REVIEW OF FLUORIDE EXPOSURE AND NEURODEVELOPMENTAL AND COGNITIVE HEALTH EFFECTS*

Revised September 16, 2020

*The September 6, 2019 draft monograph was peer reviewed by a committee convened by the National Academy of Sciences, Engineering, and Medicine (NASEM). This current draft incorporates changes in response to that review and is being submitted to the same NASEM committee for an additional round of peer review.

Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTICE

This DRAFT Monograph is distributed solely for the purpose of pre-dissemination peer review under the applicable information quality guidelines. It has not been formally disseminated by NTP. It does not represent and should not be construed to represent any NTP determination or policy.

Slide 5

Trial Phase 2

- October 2022, Court took case out of abeyance
- January - February 2024, 10-day bench trial
- September 2024, Court issued order finding Plaintiffs met burden that community water fluoridation at 0.7 mg/L presents unreasonable risk of injury to health
- January 2025, EPA appealed decision


Case 3:17-cv-02162-EMC Document 445 Filed 09/24/24 Page 1 of 80

UNITED STATES DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

FOOD & WATER WATCH, INC., et al.,
Plaintiffs,
v.
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, et al.,
Defendants.

Case No. 17-cv-02162-EMC

FINDINGS OF FACT AND CONCLUSIONS OF LAW

I. INTRODUCTION

In 2016, Congress amended the Toxic Substances Control Act ("TSCA"), empowering United States citizens to petition the Environmental Protection Agency ("EPA") to consider whether a chemical presents an unreasonable risk of injury to health. *See* Pub. L. No. 114-182, 114th Congress (Frank R. Lautenberg Chemical Safety for the 21st Century Act) (the "Act"). The Act addresses the modern-day reality that "humans beings and the environment are being exposed each year to a large number of chemical substances and mixtures." 15 U.S.C. § 2601(a)(1), and that, "among the many chemical substances and mixtures which are constantly being developed and produced, there are some whose manufacture, processing, distribution in commerce, use, or disposal may present an unreasonable risk of injury to health or the environment." *Id.* § 2601(a)(2).

To this end, under TSCA, as amended by the Act ("Amended TSCA"), a citizen is entitled to judicial review of the EPA's denial of the citizen's petition, wherein a court considers whether the chemical poses an unreasonable risk *de novo*, i.e., without deference to the EPA's decision. *See id.* § 2620(b)(4)(B). Amended TSCA sets up a system of judicial review that is remarkably different from the usual scope of judicial review of administrative actions under the Administrative Procedure Act, which confers substantial deference to administrative agencies. *See id.* Under Amended TSCA, the Court owes no deference to the EPA in assessing the risk posed by chemical substances. *See id.* If the Court finds more that the chemical at issue presents

Slide 6

TSCA Risk Evaluation

TSCA risk evaluation is comprised of the following steps:

Step 1: Hazard assessment (including hazard identification and quantitative dose response analysis);

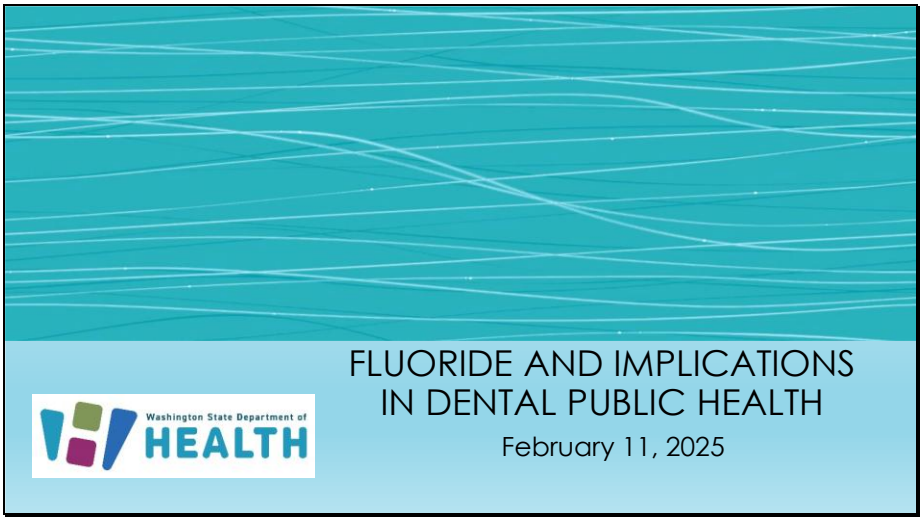
Step 2: Exposure assessment;

Step 3:Rrisk characterization. A risk evaluation under the Amended TSCA includes the three aforementioned steps of a risk assessment, as well as a fourth and final step:

Step 4: Risk determination. The "risk assessment" is the scientific technical evaluation, encompassing the first three parts of this process, resulting in an unbiased, transparent, and reproducible description of the risk.

February 11; Shelley Guinn; Oral health and fluoride

Slide 1



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Slide 3

Outline:

1. Dental Health: Tooth Development and Mineralization
2. Dental Caries - Cariology
    1. Etiology of tooth decay
    2. Burden of the Disease and Disparities
3. Fluorides and Fluoridation – Mechanisms of Action and Efficacy
    1. Systemic/Topical Considerations
    2. Fluoride intake
4. Public Health Considerations

Slide 4

ORAL HEALTH: THE DENTITION

Slide 5

Oral Health is Essential to Health and Well-Being

For the individual, for the family, for the community, and the broader economy

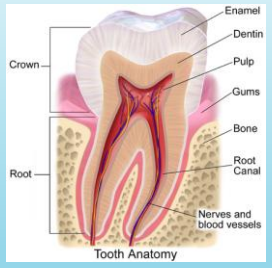
- Function
- QoL
- Self Esteem/mental health
- Employment
- Sleep
- School/Learning
- Systemic/Chronic Disease Connections
- National Security – Recruits must have functioning, disease-free dentition

Slide 6

Tooth Histology

Tooth Enamel = Covers the crown of the tooth;
Hardest substance in the body
Dentin: Lies beneath the enamel, more porous
Cementum: covers the root surfaces, less "hard," more easily demineralized

Enamel and Dentin:
Primary mineral is Hydroxyapatite, which is a crystalline calcium phosphate:
$Ca_{10}(PO_4)_6(OH)_2$



Tooth Anatomy

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DENTAL CARIES: A MULTI FACTORIAL DISEASE

Slide 8

Caries Initiation and Development

Four Factors Required
1. Susceptible tooth surface
2. Caries-causing bacteria
3. Suitable carbohydrate source
4. Time

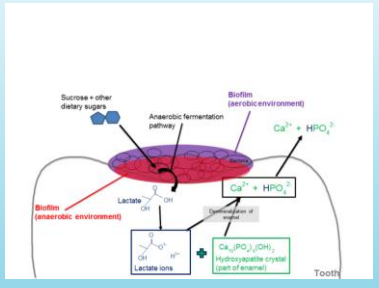
Modifying Factors
1. Saliva
2. Fluoride

Slide 9

Etiology of Tooth Decay

Tooth enamel = Crystalline Calcium Phosphate $Ca_{10}(PO_4)_6(OH)_2$

- Bacteria metabolize carbohydrates and produce destructive acids which sit undisturbed on the tooth surfaces, in grooves and pits

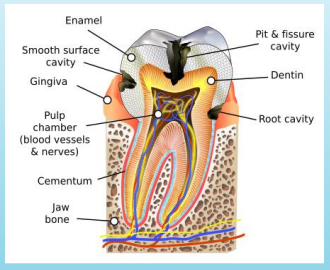


- Acids cause degradation of the mineral bonds, breaking down tooth surface and releasing **free-floating positive Ca^{2+} ions** into the oral environment.

Slide 10

Progression of Tooth Decay

1. Holes (cavities) develop in the tooth surfaces
2. Bacteria and acids may invade the nerve center
3. Pain, inflammation, infection
   Loss of function



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Dental Caries: Burden of the Disease

One of the most common non-communicable diseases affecting both adults and children globally

- Disproportionately affects the poor, the young, minority populations, and children living below 100% of the poverty level.
- Children with poor oral health are more likely to miss school and suffer academically
- US: 25% of young poor and minority children experience 80% of the disease burden
- WA: 45% of Head Start/ECEAP pre K were affected in 2016; with nearly half having rampant decay (6+ teeth)
- Early childhood caries is the single greatest risk factor for caries in the permanent dentition

(Sources: IADR, 2022; Ran, 2016; Smile Survey, 2017)

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Dental Caries: Burden of Disease

- U.S. Economic Impact of dental caries on individuals and society in 2013:
  - $111 billion
- Emergency Departments
  - WHA, 2011: Dental Pain #1 CC among uninsured
- Socioeconomic inequalities that exist in oral health at global and regional levels are detrimental to improving population oral health
  - Dentistry is inadequate for reducing the global or regional disease burden

Investment into upstream, integrated, population-wide policies would maximize oral health improvement

Slide 13

Dental Caries: A Preventable Disease

Dental caries is a chronic disease that <u>can be prevented,</u> but once it begins, can only be *managed*. A filling does not cure the disease.

Prevention and management of disease includes three levels:

I. Primary prevention:

- Behavior modifications: diet, personal hygiene, use of fluorides

II. Secondary prevention:

- Dental sealants, fluoride varnish, more frequent dental visits

III. Tertiary prevention: Control/Management of the Disease:

Silver Diamine Fluoride (SDF) – Complex Restorative procedures

Slide 14

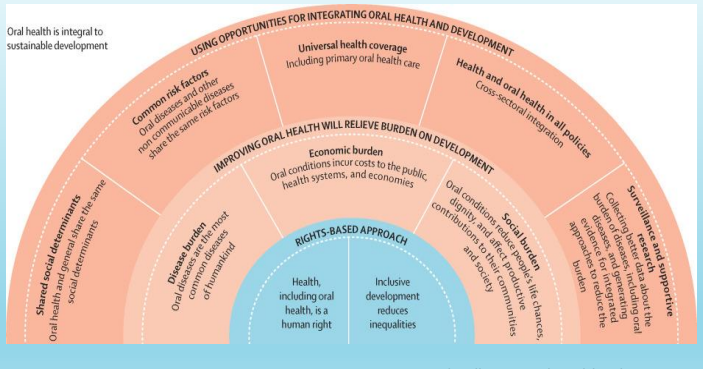


Image: Benzian and Williams, Oral Health Atlas, 2019

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FLUORIDES AND FLUORIDATION

Slide 16

Fluorides: 2 Main Mechanisms of Action

1. Reduced enamel solubility

2. Reversal of (early) caries process



The mechanisms of fluoride action are both topical <u>and</u> systemic

Slide 17

Systemic VS Topical Fluorides: Mechanism of Action

Systemic

Incorporates into dental apatite crystals during tooth development, reducing solubility of enamel: $Ca_{10}(PO_4)_6(F)_2$

- Rx Fluoride Drops/Tabs
- CWF
- Foods and beverage consumption

(These also provide a topical benefit)

Topical

Provides temporary ambient fluoride to the oral environment to assist with acid challenges (CaF_2)

- Toothpaste (1000-1100 mg/L, 1.3 mg/quarter teaspoon)
- Mouthrinse
- Dental Provider Applications
  - FL Varnish, gels
  - *Silver Diamine Fl (SDF)

Slide 18

Anti-caries effect: Pre-eruptive or Post-eruptive?

Pre-Eruptive:

- Important and effective for pits and fissure morphology (1970s)
- Singh, et. al: Maximum caries preventive effects of CWF were achieved with both "high" pre-and post-eruption exposures
- Cho, et.al, found the effect of ceased CWF in South Korea indicated that 11-year old children with approx. 4 years of CWF since birth, before CWF cessation had significantly lower DMFT ratios relative to those children who grew up in the non-fl community
- Iida and Kumar: Large national dataset of US school children, using fluorosis as a biomarker for pre-eruptive fl exposure and dental caries in first permanent molars. Findings: teeth with fluorosis consistently had lower caries experience than molars without fluorosis in both fluoridated and non-fluoridated communities

Slide 19

Anti-caries effect: Pre-eruptive or Post-eruptive?

The effects of pre- and post-eruptive fluoride complement each other

- Lifespan consideration:
  - Fluoride incorporated into developing enamel mineral may offer initial resistance to caries initiation or delay the formation of clinically detectable caries, especially at surfaces where post-eruptive fluoride is less than effective (pits, fissures, grooves)
  - Daily lower levels of topical fluoride helps to keep free fluoride available ongoing in the oral environment during times of lowered pH

Slide 20

Fluoride Intake

- Children ages 6 months to 14 years: Drinking water accounts for 40% to 70% of total fluoride intake (Public Health Reports, 2015)
- Accidentally swallowing toothpaste accounts for ~20% of total fluoride intake in very young children 1 to 3 years old.
- Adults: Drinking water provides 60% of total fluoride intake
- Other major contributors are commercial beverages and solid foods
- Most toothpastes in US contain fluoride in the form of sodium fluoride or monofluorophosphate.
- Drinking Fl water keeps a low level of fluoride (0.7 mg/L) in the mouth all day.
- FL toothpastes provide much higher concentration at important times of the day such as bedtime
- Gels used by dentists: applied 1-4 times/year and can lead to ingestions of 1.3 to 31.2 mg of fluoride each time.

Slide 21

Public Health Perspective

Communities that cease water fluoridation generally see more cavities in kids, with higher dental bills (Medicaid):

Israel halted CWF in 2014: Kids age 3 – 5 required twice as many dental procedures compared with before.

Canada: Compared Calgary and Edmonton after fluoridation cessation in Calgary, capturing children born after cessation. Findings: Decay rates increased significantly, well above Edmonton's rates. Calgary City Council voted to restart CWF

Alaska: Retrospective comparison: Juneau (stopped fl in 2007) and Anchorage. Findings: Before Juneau stopped CWF, the avg cost to treat tooth decay was similar to Anchorage. After, costs in Juneau jumped by 47%, while treatment costs in CWF Anchorage increased only 5%

Slide 22

Public Health Perspective

Slade, et. al (JDR, 2018): Cross-sectional study: For every 100 children with access to fluoridated water, there are 130 fewer decayed surfaces of primary teeth and 30 fewer decayed surfaces of permanent teeth.

Public health researchers expect the brunt of fluoride removal to fall on people with low incomes, pre-existing dental conditions, or physical or cognitive disabilities. Marginalized people tend to have fewer prevention and treatment options for tooth decay. Socioeconomic barriers can make it difficult for a person to regularly brush and floss their teeth, maintain a healthy diet or access dental care.

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CWF is Equitable Disease Prevention

- Higher caries experience is found in populations from lower SES backgrounds, and are less likely to be treated for the disease
- CWF allows fluoride to be passively delivered to community residents regardless of SES status of ability to access dental services.
- Prescription fluorides need to be ordered, monitored closely, parents forget to administer, need to be refilled monthly, may have barriers.
- Fluoride products are not always readily available to everyone in the community due to costs or availability

Slide 24

CWF: Narrowing the Disparity Gap

Research is mixed and still emerging on evidence for CWF reducing inequalities.

- The York review: Some evidence for reduced SES inequalities in caries levels in children
- Cochrane review found insufficient evidence
- National Health and Medical Research Council Review: 'found that water fluoridation reduces tooth decay by 26-44% in children, teenagers and adults' and concluded that there was limited evidence that fluoridation reduced SES inequalities and called for further high-quality research

Slide 25

CWF: Narrowing the Disparity Gap

More recent studies from different countries report evidence that fluoridation reduced SES inequalities:

- **Neidell, et. al**: *Association between community water fluoridation and adult tooth loss.* Am J Public Health (2010).
- **Kumar, et. al**: *Geographic variation in Medicaid claims for dental procedures in New York State: Role of fluoridation under contemporary conditions.* Public Health Reports (2010).
- **Elmer, et. al**: *An alternative marker for the effectiveness of water fluoridation: hospital extraction rates for dental decay, a two-region study.* Br Dent J. (2014)

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Questions?

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March 11; Claire Nitsche; Cochrane Review

Slide 1



Washington State Department of HEALTH

COCHRANE REPORT FINDINGS:

Water Fluoridation for the Prevention of Dental Caries (Review) – 2015 and 2024

Slide 2

Presenter Intros



Kyle Yomogida (he/him)
EIS Officer – WA DOH Fellow
Office of Health and Science
WA DOH



Claire Nitsche (she/her)
Environmental Health Educator
Office of Public Affairs and Equity
WA DOH

Washington State Department of Health | 2

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Background

- Dental caries impacts 60%-90% of school children in most industrialized countries.
- Community Water Fluoridation (CWF) is practiced in ~25 countries globally.
- Pre-1975, CWF was well justified and supported due to large effect sizes and lack of other sources of fluoride for populations.
- More recently, widespread benefit has been called to question due to many contemporary sources of fluoride (toothpaste, dental care, etc.)

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Cochrane Reports: Overview

2015

Meta-analysis of 155 total studies.

- 20 studies total on dental caries (71% conducted prior to 1975).
- 135 studies total on dental fluorosis (90% conducted after 1975).

2024

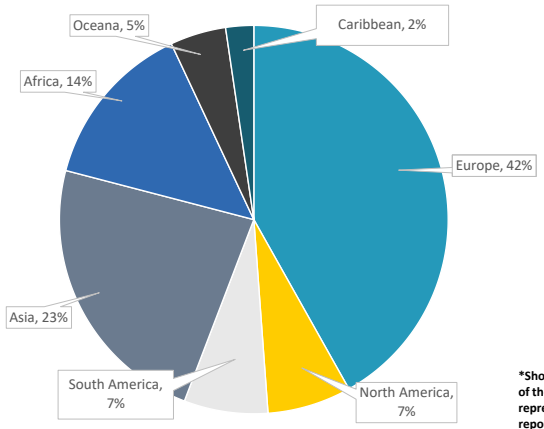
Meta-analysis of 157* total studies.

- 22 studies total on dental caries (21 on CWF initiation, 1 on CWF cessation)
- 135 studies total on dental fluorosis (90% conducted after 1975).

*Includes the 155 studies from the 2015 report

Slide 5

Continent/Regions Represented in Cochrane Report*



*Shown as the percentage of the 157 studies represented in the 2024 report.

Slide 6

Cochrane Review – Report Objectives

Objective #1

Evaluate the effects of starting or stopping CWF programs on the prevention of dental caries.

Objective #2

Evaluate the association of water fluoridation (artificial or natural) with dental fluorosis.

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Cochrane Review: Search Methodology and Inclusion Criteria

Study Search Methodology

- CENTRAL, MEDLINE, Embase, Cochrane Oral Health's Trials Register, ProQuest, Web of Science Conference Proceedings, ZETOC Conference Proceedings, US NIH Ongoing Trials Register, WHO Clinical Trials Registry Platform.
- No language, publication year, or publication status restrictions.

Inclusion Criteria – Objective #1 (Dental Caries)

- **Prospective** controlled studies comparing populations receiving water fluoridation to those receiving non-fluoridated OR naturally low-fluoridated water.
- Populations of all ages included, but the 21 studies on CWF initiation only measured caries in children (age 3-18).
- Selected studies that measured caries both within **3 years** of change in fluoridation status, and at end of study follow up.

Inclusion Criteria – Objective #2 (Dental Fluorosis)

- Any study design with concurrent control groups comparing populations exposed to different water fluoridation concentrations.
- Did not look for or include new dental fluorosis data in 2024 report.

All 157 studies in the 2024 report used non-randomized designs.

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Fluorosis

Slide 9

Dental fluorosis outcomes measured

- Dental fluorosis of aesthetic concern
  - Dean's Fluorosis Index
  - Tooth Surface Index of Fluorosis (TSIF)
  - Thylstrup and Fejerskov Index (TFI)
  - Developmental Defects of Enamel (DDE)
- Any level of dental fluorosis
- Other possible adverse effects*
- Reported fluorosis outcome data two ways:
  - All fluoride levels
  - Fluoride levels below 5 ppm

Slide 10

Dental fluorosis findings

Findings the same as 2015 Cochrane Review; no new studies added

- No new studies were added
- 2015 review is an update of the McDonagh 2000 review

Adding fluoride to water supplies increases number of people with any dental fluorosis*

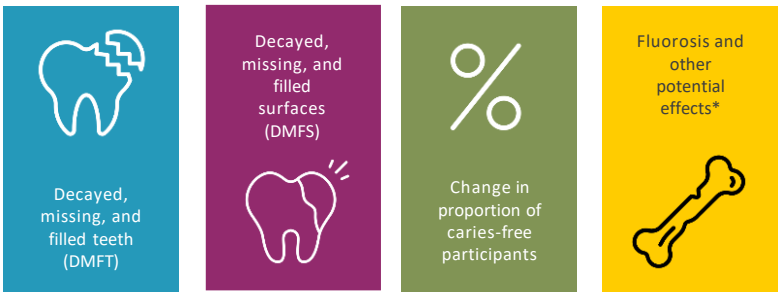
- 9% estimated prevalence of dental fluorosis of aesthetic concern (McDonagh 2000)
- 12% dental fluorosis of aesthetic concern (Cochrane 2015)

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Dental Caries and Tooth Decay

Slide 12

Dental caries outcomes measured



Slide 13

Main findings: dental caries/decay

Adding fluoride to water associated with slightly less tooth decay in children's baby teeth.

- Based on studies conducted after 1975
- Children only in studies
- Could not differentiate whether CWF reduced tooth decay in permanent teeth (DMFT) or decay on the surface of teeth (DMFS)
- Effect sizes varied for different populations

Slide 14

Inconclusive results: dental caries/decay

Could not evaluate the effect of CWF cessation on a community

- Only 1 CWF cessation study included

Could not evaluate differences in CWF effect between richer and poorer people

- Studies after 1975 were not designed to measure this relationship
- "Lack of evidence to demonstrate an effect does not equate to lack of effect."

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Limitations and Considerations

- CWF concentrations in other countries are generally higher than in the U.S. (0.7 mg/L)
- Objective was not to compare fluoride sources efficacy
  - Modes of exposure and reported benefits differ between pre-eruptive/systemic fluoride intake and post-eruptive/contact use
- Pre-eruptive benefits were not evaluated
- The inclusion criteria are strict
  - Review contains most robust study designs but is a subset of all literature
- The included studies measured caries at baseline, prior to CWF initiation or cessation, and at the end of the study period

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Some notable cessation studies were not included







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2024 Cochrane Key Takeaways

Dental fluorosis

- The authors estimate that about 40% of people may have fluorosis of any level
- At 0.7 mg/L, estimate about 12% of people have fluorosis of cosmetic concern

Tooth decay and caries

- Contemporary evidence (post-1975) do not show as clear and important effect of CWF for prevention of tooth decay in children
- Benefits are likely community- or population-specific
- CWF may still be highly effective to populations in which tooth decay is very high

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"Any initiation or cessation of a community water fluoridation programme should be fully evaluated using robust methods to address confounding, and should collect cost data to inform economic evaluation…"

"… If one of the key aims of community water fluoridation is to reduce oral health disparities, then full evaluations of the effects of community water fluoridation by socioeconomic status should be undertaken and fully reported whenever schemes are introduced or removed"

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Broader Discussion

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Questions Not Addressed By Report:
Health Promotion and Behavior Interventions

- Where else (and how often) are populations accessing fluoride in their day-to-day movements?
- What other health promotion programs are available for fluoride that may be impacting findings?
- Were other programs on alternative fluoride access run utilizing the best health education and health behavior change theory applications?
- There is no such thing as "the general public" – how does CWF compare to other interventions at a community-specific level?

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Remaining Epidemiological Questions



Characterize the total fluoride intake across populations



Study fluoride dose-response relationship to various outcomes by age and route of exposure



Community-specific evaluation of dental care access



Population-level risk/benefit analysis (costs, outcomes, knock-on effects)

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Examples of Informative Future Studies

Total fluoride consumption relationship to specific health outcomes

- Prospective cohort design
- Characterize fluoride intake from different sources
- Urinalysis to verify total intake
- Could separate groups by CWF initiation/cessation

Water district-specific risk/benefit analysis

- Account for demographics (e.g., age)
- Include covariates like dental care access and insurance coverage coverage
- Cost of care
- Cost of CWF
- Predictive model potential health effects (positive or negative)

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Thank you!



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SUPPLEMENTAL SLIDES

Slide 26

Why the defined follow up period matters


ORIGINAL ARTICLE

Fluoridation cessation and children's dental caries: A 7-year follow-up evaluation of Grade 2 schoolchildren in Calgary and Edmonton, Canada


RESEARCH ARTICLE

Consequences of community water fluoridation cessation for Medicaid-eligible children and adolescents in Juneau, Alaska


Systemic effect of water fluoridation on dental caries prevalence

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Dental caries outcomes measured

- Change in number of decay, missing, and filled primary and permanent teeth (DMFT)
- Change in the number of decay, missing, and filled primary and permanent tooth surfaces (DMFS)
- Change in the proportion of caries-free participants
- Adverse effects including fluorosis and potential effects* like skeletal fluorosis, hip fractures, cancer, congenital malformations, mortality

**There is lack of evidence for these harms, but authors chose to include in acknowledgement of growing interest (See McDonagh 2000, NHMRC 2017)*

Slide 28

Inconclusive results: dental caries/decay

Could not evaluate the effect of CWF cessation on a community

- Only 1 CWF cessation study included

Could not evaluate differences in CWF effect between richer and poorer people

- Studies after 1975 were not designed to measure this relationship

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Remaining Epidemiological Questions

- Characterize the cumulative F- intake across populations
- Fluoride dose-response relationship to various outcomes; need better exposure contrast rather than high/low water systems
- Community-specific evaluation of dental care access
- Quantifying the risk of one population versus the benefits to another
  - Community-specific risk (e.g., age of population)
  - Consider dental care access and affordability (e.g., many dentists do not accept Medicaid and Medicare)
  - Consider systemic costs

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Dental fluorosis findings

- Findings the same as 2015 Cochrane Review; no new studies added
- Adding fluoride to water supplies increases number of people with dental fluorosis

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Pre-eruption CWF studies

- The Pre-and Posteruptive Effects of Fluoride in the Caries Decline (Beltran 1988)
- Clinical Evidence of the Role of Pre-eruptive Fluoride in Caries Prevention (Thylstrup 1990)
- Systemic versus topical fluoride (Hellwig 2004)
- Fluoride in Caries Prevention: Is the Effect Pre- or Post-eruptive? (Groeneveld 1990)
- Relative Effects of Pre- and Posteruption Water Fluoride on Caries Experience of Permanent First Molars (Singh 2002)
- Effects of water fluoride exposure at crown completion and maturation on caries of permanent first molars (Singh 2007)

March 25; Herbie Duber; Case Studies

Slide 1



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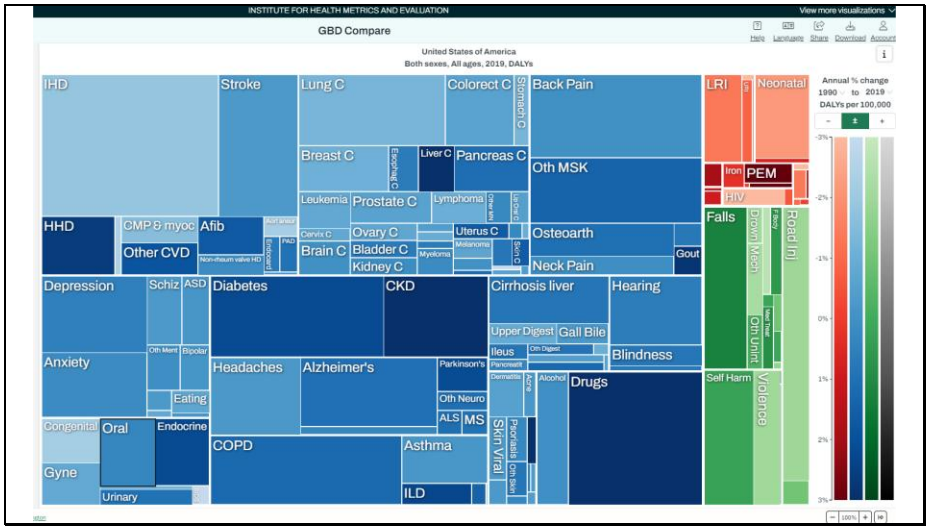
COMMUNITY WATER FLUORIDATION - CASE STUDIES

Slide 2

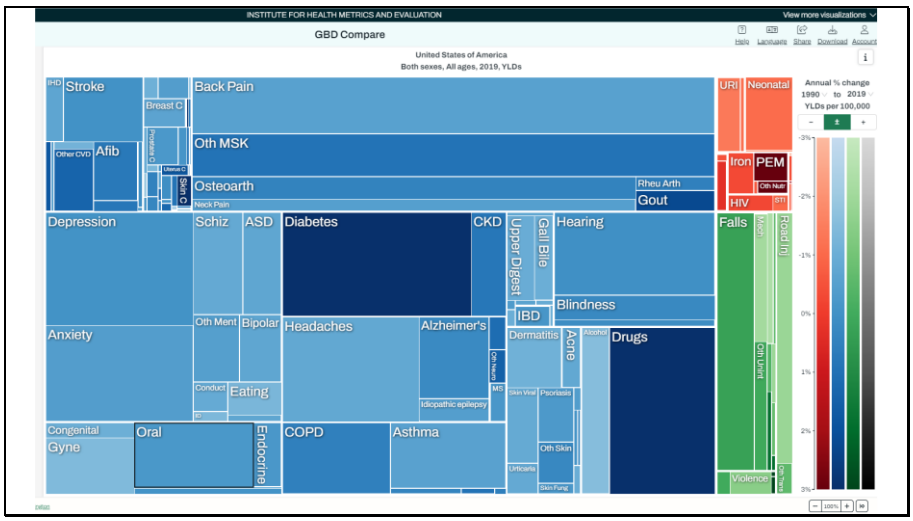
Overview

Brief overview of BoD and CWF
CWF and the Cochrane Review
Calgary, AB
Juneau, AK
Conclusions

Slide 3

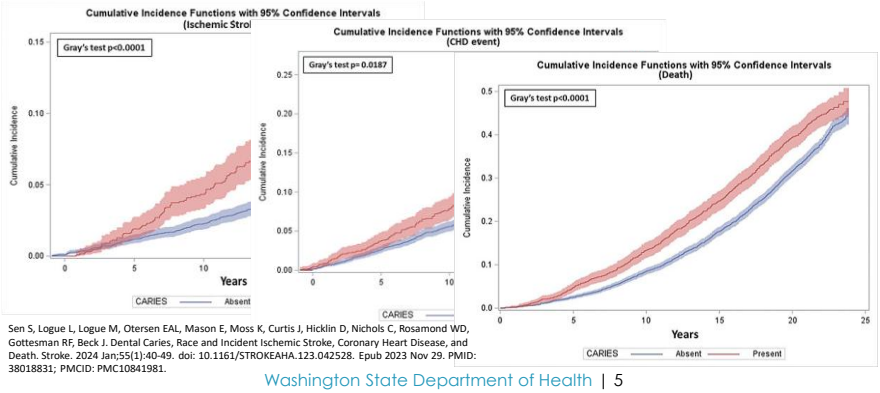


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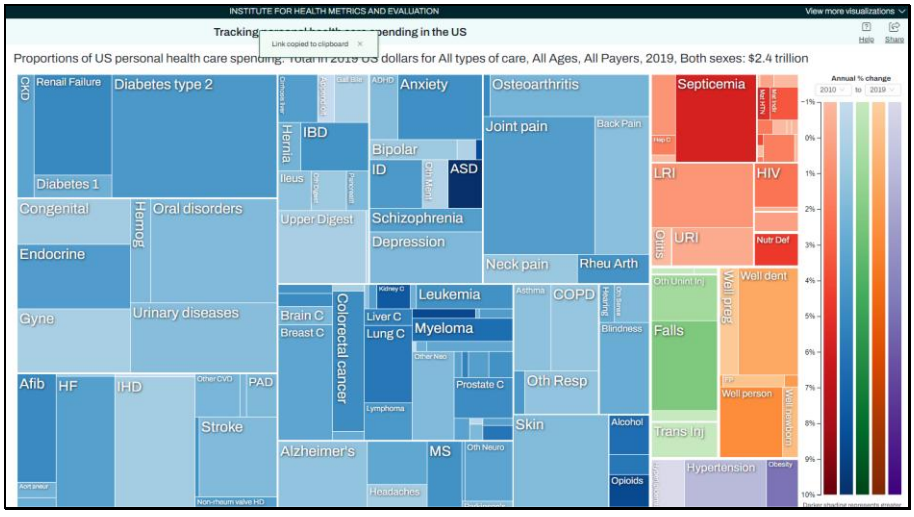
Slide 5

Association between dental caries and stroke, CHD and death



Sen S, Logue L, Logue M, Otersen EAL, Mason E, Moss K, Curtis J, Hicklin D, Nichols C, Rosamond WD, Gottesman RF, Beck J. Dental Caries, Race and Incident Ischemic Stroke, Coronary Heart Disease, and Death. Stroke. 2024 Jan;55(1):40-49. doi: 10.1161/STROKEAHA.123.042528. Epub 2023 Nov 29. PMID: 38018831; PMCID: PMC10841981.

Slide 6



Slide 7

Community Water Fluoridation

Tenth Year of the Grand Rapids-Muskegon Study

Effect of Fluoridated Public Water Supplies on Dental Caries Prevalence

By FRANCIS A. ARNOLD, Jr., D.D.S., H. TRENDLEY DEAN, D.D.S., PHILIP JAY, D.D.S., and JOHN W. KNUTSON, D.D.S., Dr.P.H.

- Introduced in 1945 in Grand Rapids, MI
  - 10yr follow-up study that compared Grand Rapids to Muskegon, MI
  - 60% reduction in rate of caries (primary and permanent) among children born after introduction of CWF
  - "the percentage of children classed as having fluorosis has increased, but, as anticipated, this increase is confined to the milder forms. As pointed out previously (7), the signs of the milder forms of fluorosis caused by ingestion of water containing 1 p.p.m. fluoride as a rule do not appear on the anterior teeth. "

Slide 8

Community Water Fluoridation

Table 2. Average number of def[1] deciduous teeth per child[2] in Grand Rapids and Muskegon, Mich., by year of examination

Age last birthday	Basic examinations, 1944–45	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
Grand Rapids, Mich.											
4	4.19	5.40	3.43	3.19	3.02	2.75	2.46	2.13	2.17	2.06	2.12
5	5.37	6.15	5.08	3.89	4.03	3.27	2.90	2.27	2.32	2.29	2.50
6	6.43	6.98	5.73	5.38	4.78	4.59	3.73	2.98	2.93	2.92	2.95
7	6.29	7.66	6.11	5.84	5.20	4.83	5.72	4.03	3.48	3.10	3.26
8	5.78	8.00	5.10	5.07	4.88	4.75	4.91	4.12	3.89	3.48	3.31
9	4.59	.....	4.45	4.11	4.43	4.41	4.23	3.86	3.66	3.35	3.00
10	2.84	.....	2.84	3.16	3.06	2.86	2.36	2.43	2.61	2.38	2.35
11	1.35	.....	2.12	2.78	1.77	1.19	1.16	1.35	1.51	1.90	1.32
12	.47	.28	.13	.11	.25	.10	.35	.25	.30	.34	.44
13	.18	.13	.14	.14	.17	.10	.15	.12	.17	.19	.18
Muskegon, Mich.[3]											
4	5.05	.....	3.44	4.67	4.39	4.41	5.32	4.46	4.35	3.44	3.03
5	6.82	.....	5.86	5.65	5.55	5.56	5.65	5.25	5.39	4.42	3.98
6	7.17	.....	6.24	6.18	6.06	5.99	6.02	5.67	5.75	5.71	4.83
7	6.66	.....	6.83	5.95	6.92	6.33	5.83	5.77	5.67	5.46	5.35
8	6.06	.....	4.83	3.85	4.80	6.08	5.06	5.32	5.28	4.95	4.98
9	4.89	.....	4.32	4.34	4.71	4.48	4.09	4.17	4.36	4.29	3.81
10	3.08	.....	3.15	3.67	2.79	2.77	3.49	2.86	2.69	2.96	2.75
11	1.33	.....	1.67	2.90	.64	1.21	1.09	1.46	1.20	1.38	1.42
12	.42	.....	.14	.37	.64	.68	.61	.31	.54	.32	.61
13	.23	.....	.29	.17	.11	.11	.13	.15	.08	.21	.12

[1] Decayed, extraction indicated, or filled deciduous teeth. A decayed and filled tooth is counted only once.
[2] See table 1 for small numbers involved in some instances.
[3] The basic examinations in Muskegon were not done until late spring of 1945; therefore, no examinations were made in the fall of 1945.

Table 3. Average number of DMF[1] permanent teeth per child[2] in Grand Rapids and Muskegon, Mich., by year of examination

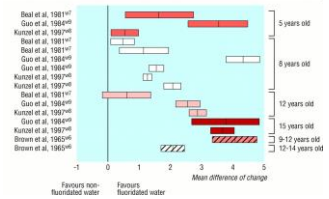
Age last birthday	Basic examinations, 1944–45	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954
Grand Rapids, Mich.											
6	0.78	0.56	0.23	0.37	0.26	0.38	0.26	0.26	0.23	0.12	0.19
7	1.89	1.72	1.11	1.09	1.04	.76	1.03	.84	.90	.71	.69
8	2.95	3.27	2.54	2.62	2.30	2.16	1.77	1.58	1.50	1.41	1.27
9	3.90	.....	2.98	3.12	2.67	2.48	2.36	2.04	2.02	1.83	1.97
10	4.92	.....	3.70	3.56	3.51	3.56	3.17	2.93	2.71	2.41	2.34
11	6.41	.....	4.24	3.56	4.32	4.69	4.36	3.67	3.49	3.12	2.98
12	8.07	9.53	7.62	7.03	8.32	7.02	7.10	5.89	5.04	4.76	3.87
13	9.73	10.76	8.92	8.47	8.34	8.11	7.21	6.60	5.87	5.12	5.05
14	10.95	11.90	9.41	9.50	9.41	8.90	8.55	8.21	7.23	5.92	6.78
15	12.48	12.68	11.26	11.94	10.61	11.80	10.12	8.91	9.04	9.75	8.67
16	13.50	13.00	9.33	12.47	13.50	11.83	11.35	11.06	10.14	9.33	9.95
Muskegon, Mich.[3]											
6	0.81	.....	0.48	0.66	0.79	0.63	0.73	0.80	0.52	0.35	0.45
7	1.99	.....	1.33	1.05	2.19	1.43	2.01	1.88	1.66	1.24	1.14
8	2.81	.....	2.83	2.15	3.50	2.58	2.96	2.63	2.49	2.66	2.18
9	3.81	.....	3.29	3.54	3.58	3.88	3.89	3.52	3.65	3.22	3.16
10	4.91	.....	4.27	3.60	4.87	4.44	4.53	4.32	3.90	3.64	3.72
11	6.32	.....	4.25	4.70	4.71	5.93	5.67	5.34	5.04	4.79	4.48
12	8.66	.....	8.43	6.79	7.82	7.21	6.88	7.71	7.00	6.53	6.12
13	9.98	.....	9.02	9.23	10.52	9.52	9.58	9.36	8.71	8.20	7.98
14	12.00	.....	11.09	12.00	12.27	11.08	12.11	11.36	10.06	10.33	10.74
15	12.96	.....	11.17	12.89	12.66	10.32	10.94	12.38	11.57	11.69	11.19
16	14.07	.....	19.00	12.77	14.31	12.51	13.91	13.16	12.36	11.48	12.55

[1] Decayed, missing, or filled permanent teeth. A decayed and filled tooth is counted only once.
[2] See table 1 for small numbers involved in some instances.
[3] The basic examinations in Muskegon were not done until the late spring of 1945; therefore, no examinations were done in the fall of 1945.

Slide 9

Community Water Fluoridation

- MANY studies since conducted demonstrating the effectiveness of CWF
  - 26-44% reduction in tooth decay in children and 27% in adults in Australia (Slade et al 2013)
  - Children in England found to have significant improvement in decayed, missing and filled teeth for primary/permanent teeth (BMJ Systematic Review 2000)



Change in decayed, missing, and filled teeth for primary/permanent teeth (mean difference and 95% confidence interval)

Slide 10

Community Water Fluoridation

In 2022, More than 209 million Americans (>70% of the population) had access to community water fluoridation (https://www.cdc.gov/fluoridation/php/statistics/2022-water-fluoridation-statistics.html)

CDC Scientific Statement on Community Water Fluoridation



The U.S. Community Preventive Services Task Force, on the basis of systematic reviews of scientific literature, issued a strong recommendation in 2001 and again in 2013, for community water fluoridation for the prevention and control of tooth decay.

Slide 11

Community Water Fluoridation – Cochrane Review

- Affirms that fluoride reduces tooth decay in children and adults.
- Cochrane review does NOT suggest that CWF is ineffective
  - Raises important questions about where people receive fluoride since 1970 and the ADDED VALUE of CWF at a population level
- The review was NOT designed to evaluate CWF and its impact on dental health inequities

Slide 12

Calgary, AB

- Fluoride was introduced in Calgary's drinking water in 1991
- Fluoridation was stopped in 2011
- Fluoride was reintroduced in 2021
  - Naturally occurring fluoride at concentrations from 0.1 to 0.4 mg/L
  - City adjusts fluoride to maintain a concentration of 0.7mg/L
- *The infrastructure costs to reintroduce fluoride at Calgary's two water treatment plants was $28.1M with additional annual costs of $1M for operating and maintenance at both plants. This translates into less than 10 cents per person, per month. (*https://www.calgary.ca/water/drinking-water/fluoride.html)

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Calgary, AB – Dental caries in children

- Retrospective, population-based study including children <12 undergoing GA for caries related treatment in Calgary and Edmonton
- 2659 children
- 2010-2019
- Variables collected: age, sex, dental diagnosis, neighborhood income level (three levels), facility type
- Calculated per capita rate of children requiring caries related dental treatments under GA per 10,000

Canadian Journal of Public Health (2024) 115:305–314
https://doi.org/10.17269/s41997-024-00858-w

ORIGINAL ARTICLE

Community water fluoride cessation and rate of caries-related pediatric dental treatments under general anesthesia in Alberta, Canada

Elnaz Yazdanbakhsh[1] · Babak Bohlouli[1] · Steven Patterson[1] · Maryam Amin[1]

Received: 18 May 2023 / Accepted: 19 January 2024 / Published online: 22 February 2024
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Calgary, AB - Dental caries in children

Table 2 Rate of caries-related dental treatments under GA among children aged 0–11 years per 10,000 children, stratified by year and fluoridation status

Age groups	Year	Fluoridation status		Per-capita difference
		Non-fluoridated area	Fluoridated area	
Total	2010/11	18	15	3
	2014/15	27	14	13
	2018/19	32	17	15
0–5 years	2010/11	22	18	4
	2014/15	38	17	21
	2018/19	45	24	21
6–11 years	2010/11	14	11	3
	2014/15	15	10	5
	2018/19	19	11	8

Table 3 Risk of caries-related dental treatments under GA per 10,000 children in the population, stratified by age group

	All pediatric patients OR (95% CI)	0–5-year-olds OR (95% CI)	6–11-year-olds OR (95% CI)
2010/11	1.20 (1.03–1.41)	1.21 (0.99–1.48)*	1.17 (0.89–1.53)*
2014/15	1.96 (1.70–1.26)	2.28 (1.91–2.71)	1.45 (1.13–1.86)
2018/19	1.84 (1.62–2.08)	1.93 (1.66–2.39)	1.74 (1.40–2.17)

*Statistically non-significant
Reference category: fluoridated area's per-capita rate
OR, odds ratio; *CI*, confidence interval

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Calgary. AB - Dental caries in children

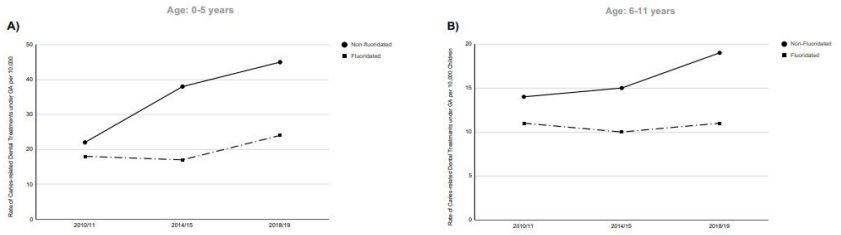


Fig. 2 Trend of caries-related dental treatments under general anesthesia per 10,000 children in fluoridated (Edmonton) and non-fluoridated (Calgary) areas, stratified by age groups: (**A**) 0–5 years and (**B**) 6–11 years

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Calgary, AB – CWF and social inequities



Canadian Journal of Public Health (2022) 113:955–968
https://doi.org/10.17269/s41997-022-00654-4

QUANTITATIVE RESEARCH

Fluoridation cessation and oral health equity: a 7-year post-cessation study of Grade 2 schoolchildren in Alberta, Canada

Lindsay McLaren[1] · Steven K. Patterson[2] · Peter Faris[3] · Guanmin Chen[1,3] · Salima Thawer[1,4] · Rafael Figueiredo[2,5] · Cynthia Weijs[1,6] · Deborah A. McNeil[1,7] · Arianna Waye[8] · Melissa L. Potestio[1]

Received: 21 June 2021 / Accepted: 16 May 2022 / Published online: 7 July 2022
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- Cross sectional population-based survey of children in Grade 2 in Calgary and Edmonton in 2009/2010, 2013/2014, 2018/2019
- Estimated association between socioeconomic indicators and dental caries indicators/untreated decay in two or more teeth
- Compared associations over time and between cities post-CWF cessation

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Calgary, AB – CWF and social inequities

- Social inequities existed in BOTH cities.
  - Odds of *untreated decay* higher among those without dental insurance in 2018/19 (Calgary OR 3.34; Edmonton OR 2.31)
  - Higher levels of *deft*, *DMFT* and *untreated decay* in lowest material deprivation tercile
- Widening gap in outcomes associated with some measures of social inequities in Calgary compared to Edmonton
  - OR of untreated decay without dental insurance
    - Calgary 2009/10: 1.79; 2013/14: 2.0; 2018/19: 3.34
    - Edmonton: 2013/14: 2.06; 2018/19: 2.31

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Juneau, AK

- City of Juneau voted to discontinue CWF in 2007. Fairbanks, AK similarly discontinued CWF in 2011. This reduced the total population with access to CWF from ~65% to ~50% between 2007 and 2014
- Meyer et al. examined the impact of CWF cessation in children and adolescents 0-18 living in families meeting Medicaid requirements.
- Retrospective comparative research design
  - Examined Medicaid dental claims in 2003 and 2012
  - Examined dental caries procedures and dental caries-related costs

Table 2 The Mean (SD) Number of Caries Procedures per Child in 2003 and 2012, and a Summary of the Bivariate and Binary Regression Analyses

Age Group (years)	*Mean (SD) 2003 Optimal CWF*	*Mean (SD) 2012 Suboptimal CWF*	*Mann-Whitney U p*	*Logistic Regression[a] Optimal CWF OR, [95% CI]*
0 to < 6	1.55 (3.89) $n = 194$	2.52 (4.35) $n = 301$	0.0001	0.488, [0.33, 0.73]
0 to < 7	2.01 (4.22) $n = 303$	2.68 (4.57) $n = 461$	0.004	0.699, [0.52, 0.95]
7 to < 13	1.61 (3.38) $n = 352$	1.64 (2.60) $n = 400$	NS	NS
13 to 18	2.75 (4.73) $n = 198$	3.04 (4.66) $n = 191$	NS	NS
0 to 18	2.02 (4.05) $n = 853$	2.35 (3.99) $n = 1052$	0.001	0.748, [0.62, 0.90]

NS Not Significant
[a]Adjusted for gender and race

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Juneau, AK

Table 3 Mean Caries-related Treatment Costs by Age in 2003 and 2012 and Adjusted for Inflation

Age Group (years)	Mean ($) 2003 Optimal CWF	Mean ($) 2012 Suboptimal CWF	Mann-Whitney U p	Total Cost Inc/ % Inc	Adjusted* –25% Inflation	Increase ($) Attributed to Suboptimal CWF
0 to < 6	272.72	644.72	0.0001	372.00/136%	111%	302.71
0 to < 7	350.13	692.87	0.0001	342.74/98%	73%	255.60
7 to < 13	241.52	382.44	0.001	140.92/58%	33%	79.70
13 to 18	519.07	795.68	0.035	276.61/53%	28%	145.34
0 to 18	344.34	593.70	0.0001	249.36/72%	47%	161.84

*Service provider charges were used rather than Medicaid reimbursement amounts for comparisons by accurately adjusting for inflation

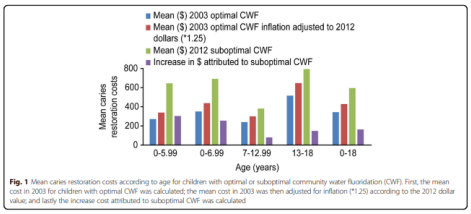


Fig. 1 Mean caries restoration costs according to age for children with optimal or suboptimal community water fluoridation (CWF). First, the mean cost in 2003 for children with optimal CWF was calculated; the mean cost in 2003 was then adjusted for inflation (*1.25) according to the 2012 dollar value; and lastly the increase cost attributed to suboptimal CWF was calculated

Slide 20

Conclusions

- Community water fluoridation has **REPEATEDLY** been demonstrated to be a highly effective public health intervention across many studies to prevent dental carries in both children and adults
  - Contemporary studies have again demonstrated this benefit
- Cochrane metanalysis has limited applicability due to its inclusion/exclusion criteria, but **NO STUDY IS PERFECT** (especially in public health)
- Detrimental effects are NOT the focus of this discussion, but a ***critical*** part of the overall discussion related to CWF
  - Causation vs. Association
  - Probable mechanism of action
  - Defining a gradient of potential benefit and potential harm at various CWF concentrations is essential

Absence of evidence ≠ Evidence of absence

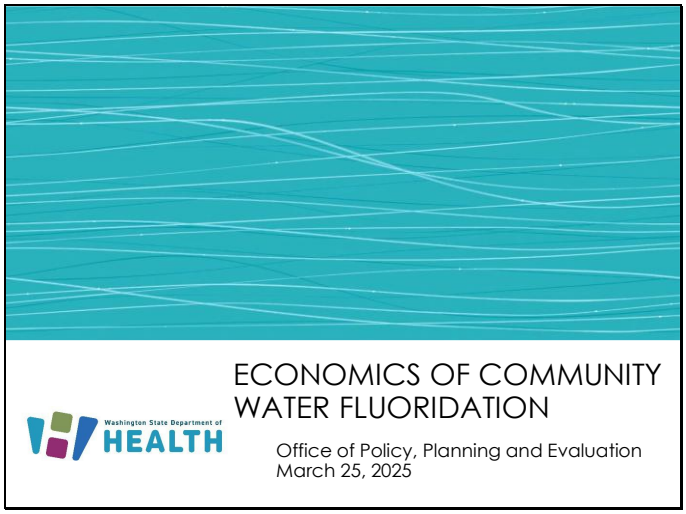
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Washington State Department of HEALTH

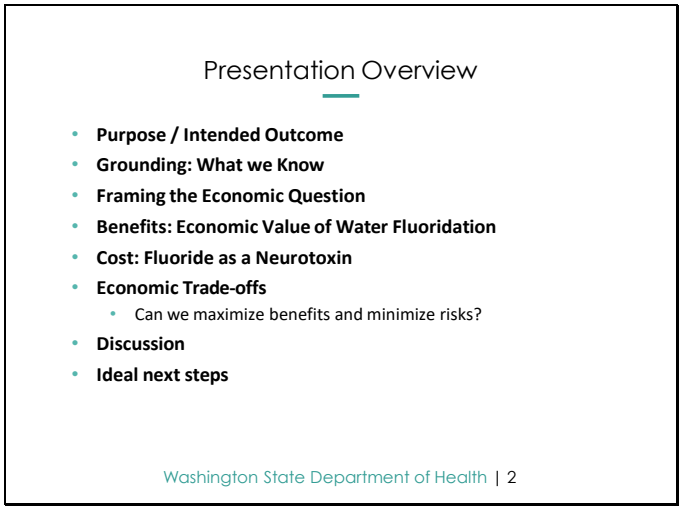
To request this document in another format, call 1-800-525-0127. Deaf or hard of hearing customers, please call 711 (Washington Relay) or email civil.rights@doh.wa.gov.

March 25; Anna Hidle; Economic Analysis

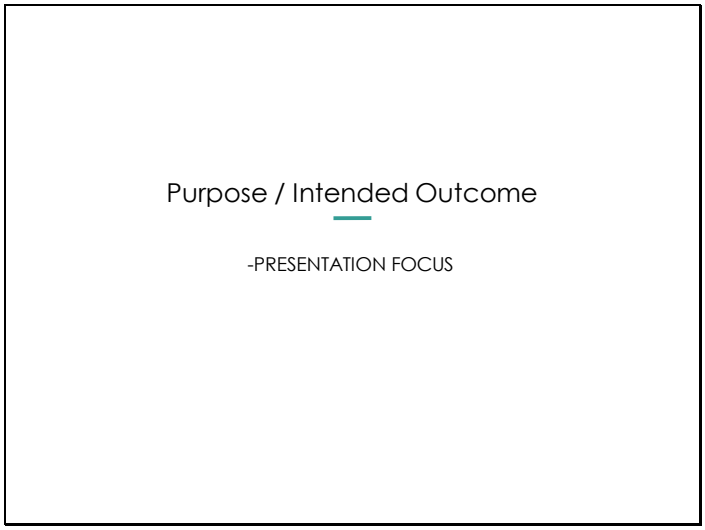
Slide 1



Slide 2



Slide 3



Slide 4

Purpose / Intended Outcome

Meeting Purpose:

- Discussion of the economic costs and benefits of community water fluoridation.

Intended Outcome:

- Shared understanding of the economic costs and benefits of community water fluoridation.

Presentation Focus:

- Economics
- Community water fluoridation

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Grounding: What we know

Slide 6

Grounding: What we know

General:

- Fluoride = Good oral health, reduces poor oral outcomes
- Topical & Systemic
- Benefit realized when fluoride encounters teeth
- 73% of U.S. drinking water is fluoridated
- Recommended water fluoridation at 0.7 mg fluoride/L

Slide 7

Grounding: What we know

Not including potential risks of fluoride (fluorosis, lost IQ points)

Economics of Fluoride:

- **Benefits of fluoridating water is documented in literature to have declined** since 1970, with the introduction of other methods of fluoride.
- Community fluoridation of water has **historically been presented in literature to be cost-effective and provide a return on investment.**
  - The **higher the incidence of tooth decay** before fluoridation and the **larger the population served**, the **greater the economic benefits**.

Slide 8

Grounding: What we know

Not including potential risks of fluoride (fluorosis, lost IQ points)

CDC reports the Economics of Fluoride:

- The savings associated with water fluoridation in communities (>1,000 people) yields an **average savings of $20 per dollar invested**. (1)
- A study of a community water fluoridation in Colorado compared program costs with treatment savings achieved through reduced tooth decay. Analysis of 172 public water systems (<1,000 people or more in each) found that **1 year of exposure to fluoridated water yielded an average savings of $60 per person** when the lifetime costs of maintaining a restoration were included. (2)
- Analyses of Medicaid claims data in 3 states (Louisiana, New York, and Texas), have also found that **children living in fluoridated communities have lower cavity related treatment costs as compared to similar children living in non-fluoridated communities; the difference in annual per child treatment costs ranged from $28 to $67**. (3,4,5)

Reference: CDC Scientific Statement on Community Water Fluoridation
(1) O'Connell et. al., Costs And Savings Associated With Community Water Fluoridation In The United States | Health Affairs. 2016
(2) O'Connell et. al., Costs and savings associated with community water fluoridation programs in Colorado. Prev Chronic Dis. 2005;2(Spec No): A06.
(3) Water Fluoridation and Costs of Medicaid Treatment for Dental Decay -- Louisiana, 1995-1996
(4) Kumar et. al., Geographic variation in Medicaid claims for dental procedures in New York State: role of fluoridation under contemporary conditions. Public Health Rep. 2010;125(5):647–654.
(5) Texas Department of State Health Services. Water fluoridation costs in Texas: Texas Health Steps (EPSDT-MEDICAID). Texas Department of State Health Services; 2000. Accessed January 31, 2024.

Slide 9

Grounding: What we know

Not including potential risks of fluoride (fluorosis, lost IQ points)

American Fluoridation Society reports the Economics of Fluoride:

- For most cities, **every $1 invested in water fluoridation saves $38** in dental treatment costs. (CDC)
- A Texas study confirmed that **the state saved $24 per child, per year in Medicaid expenditures** for children because of the cavities that were prevented by drinking fluoridated water. (Texas Dept of Oral Health)
- A 2010 study in New York State found that **Medicaid enrollees in less fluoridated counties needed 33 percent more fillings, root canals, and extractions than those in counties where fluoridated water** was much more prevalent. As a result, the **treatment costs per Medicaid recipient were $23.65 higher for those living in less fluoridated counties.** (Kumar et. al.)
- Researchers estimated that in 2003 **Colorado saved nearly $149 million in unnecessary treatment costs by** fluoridating public water supplies—**average savings of roughly $61 per person**. (Ntl Center for Biotech Info)
- A 1999 study compared Louisiana parishes (counties) that were fluoridated with those that were not. The study found that **low-income children in communities without fluoridated water were three times more likely** than those in communities with fluoridated water **to need dental treatment** in a hospital operating room. (CDC)
- By reducing the incidence of decay, fluoridation makes it less likely that toothaches or other serious dental problems will drive people to hospital emergency rooms (ERs)—where treatment costs are high. A 2010 survey of hospitals in Washington State found that **dental disorders were the leading reason why uninsured patients visited ERs.** (Washington State Hospital Association)
- Scientists who testified before Congress in 1995 **estimated that national savings from water fluoridation totaled $3.84 billion each year**. (Florida Journal of Environmental Health)

Reference: Cost-Effectiveness | American Fluoridation Society

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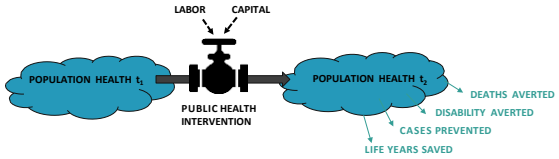
Framing the Economic Question

- LITERATURE REVIEW

Slide 11

Framing the Question: Cost Effectiveness Analysis

Compares the **net cost** of an intervention to the **changes in health outcomes**



Net cost =

$$\textit{Cost Effectiveness Ratio} = \frac{\text{Net cost}}{\text{Improvement in health produced by the intervention}}$$

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Framing the Economic Question

Intervention: Community Water Fluoridation

Includes cost of adverse effects of the intervention and other costs induced by the intervention

Costs of health outcome averted because of the intervention

Net cost =

Improvement in health produced by the intervention

$$\textit{Cost Effectiveness Ratio} = \frac{\text{Net cost}}{\text{Improvement in health produced by the intervention}}$$

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Framing the Economic Question

Intervention: Community Water Fluoridation

Intervention Cost

- Fixed costs
  - Fluoridation Facilities
- Annual costs
  - Maintenance
  - Operation (Staff cost)
  - Monitoring

Reference: Ran et. al. 2016. Economic Evaluation of Community Water Fluoridation: A Community Guide Systematic Review - PMC

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Framing the Economic Question

Not including potential risks of fluoride (fluorosis or lost IQ points)

Intervention: Community Water Fluoridation

Costs averted or Intervention Benefit*

- **Healthcare cost averted**
  - dental examination
  - **restoration**
  - extraction
  - **lifetime treatment**
- **Productivity cost averted**
  - worktime lost
  - missed school/learning
- **Other losses averted**
  - transport to dental visits

Risks

*Benefits listed in bold are common drivers of cost analysis

Reference: Ran et. al. 2016. Economic Evaluation of Community Water Fluoridation: A Community Guide Systematic Review - PMC

Slide 15

Framing the Economic Question

Intervention: Community Water Fluoridation

Costs averted or Intervention Benefit*

- **Healthcare cost averted**
  - dental examination
  - **restoration**
  - extraction
  - **lifetime treatment**
- **Productivity cost averted**
  - worktime lost
  - missed school/learning
- **Other losses averted**
  - transport to dental visits

Risks

- Fluorosis
- Loss of IQ Points

Economic Question:
How do we…
maximize the benefits of fluoride
while
minimizing the risk of fluoride as a neurotoxin?

*Benefits listed in bold are common drivers of cost analysis

Reference: Ran et. al. 2016. Economic Evaluation of Community Water Fluoridation: A Community Guide Systematic Review - PMC

Slide 16

Framing the Economic Question

Study Perspective: costs to whom

Health Care perspective

Typically includes all direct health care costs:

- Easier to value
  - Cost of intervention
  - Changes in Health Outcomes
    - Costs of morbidity and mortality

Societal perspective

Typically includes healthcare perspective plus may include:

- Easier to value
  - Out-of-pocket costs (healthcare, transportation, etc.)
  - Productivity losses (caregiver)
- More difficult to value
  - Productivity losses (non-wage earners)
  - Equity

References:
1) Resch S, Menzies N, Portnoy A, Clarke-Deelder E, O'Keeffe L, Suharlim C, Brenzel L. How to cost immunization programs: a practical guide on primary data collection and analysis. 2020.Cambridge, MA: immunizationeconomics.org/ Harvard T.H. Chan School of Public HealthtoCost_Digital_12.24.20.pdf (squarespace.com)
2) AJPH202032033_Bloom_1049_1054 (nih.gov)

Slide 17

Framing the Economic Question: Literature Review

Year	Author(s)	Title	Subject Matter	Learning(s)
2015	Ko & Thiessen	A critique of recent economic evaluations of community water fluoridation	Fluoride	Critique of previous econ evals, challenging published estimate, adds fluorosis
2022	Goodwin et. al.	Evaluation of water fluoridation scheme in Cumbria: the CATFISH prospective longitudinal cohort study	Fluoride	Clinical and cost-effectiveness of water fluoridation
2024	Moore et. al.	How effective and cost-effective is water fluoridation for adults and adolescents? The LOTUS 10-year retrospective cohort study	Fluoride	Clinical and cost-effectiveness of water fluoridation
2025	Osmunson & Cole.	Community Water Fluoridation a Cost-Benefit-Risk Consideration	Fluoride	Critique of previous econ evals, adds fluorosis and lost IQ points

Slide 18

Framing the Economic Question: Literature Review

Reviewed literature on the cost of elevated blood lead levels in children to better understand the potential costs of lost IQ points.

Year	Author(s)	Title	Subject Matter	Learning(s)
2002	Grosse et. al.	Economic gains resulting from the reduction in children's exposure to lead in the United States.	Lead	IQ points and lifetime earnings lost
2021	Boyle et. al.	Estimated IQ points and lifetime earnings lost to early childhood blood lead levels in the United States	Lead	IQ points and lifetime earnings lost

Slide 19

Benefits: Economic Value of Water Fluoridation

- NOTABLE AND RECENT COST EFFECTIVENESS LITERATURE

Slide 20

Perspective: Societal
US$: 2010

Journal Article Review

Cost Effectiveness of Community Water Fluoridation

December 2014 – Ko & Thiessen. A critique of recent economic evaluations of community water fluoridation

Focus: Examine cost-effectiveness studies of water fluoridation

Methods: Examine and propose alternative methods and underlying data from US economic evaluation on community water fluoridation.

Findings: Incorrect dental treatment costs, flawed effectiveness estimates, and overestimate of benefits. Costs to water treatment plants and communities not reflected.

Conclusion: Minimal correction reduced the savings to $3 per person per year (PPPY) for a best-case scenario, but this savings is eliminated by the estimated cost of treating dental fluorosis (minimum cost of $3.24 PPPY).

Note: Does not include cost of lost IQ points.

Reference: Ko & Thiessen. 2014. Full article: A critique of recent economic evaluations of community water fluoridation

Slide 21

Perspective: Payer
GBP: 2014

Journal Article Review

Cost-Effectiveness of Community Water Fluoridation

November 2022 – Goodwin et. al., Evaluation of water fluoridation scheme in Cumbria: the CATFISH prospective longitudinal cohort study

Question: Is the reduction of caries due to community water fluoridation and is it cost-effective?

Methods: 2 cohorts (birth cohort and children in school cohort), United Kingdom

Findings:

- Modest beneficial effect for birth cohort (4% less decay in dentine)
- **Likely cost-effective for both the birth cohort and the older school cohort** at a willingness-to-pay threshold of £20,000 per quality-adjusted life-year (sensitivity probability CE 77% & 64%)
- No significant difference in the performance of water fluoridation on caries experience across deprivation quintiles

Conclusion: Prevalence of caries and impact of water fluoridation smaller than previous studies. Effective in birth cohort. **Cost-effective.**

Note: Suggest long-term follow-up to fully understand the balance of benefits and potential risks (e.g. fluorosis) of water fluoridation in contemporary low-caries populations.

Reference: Goodwin et. al. 2022 Evaluation of water fluoridation scheme in Cumbria: the CATFISH prospective longitudinal cohort study

Slide 22

Perspective: Public Sector
US$: 2020

Journal Article Review

Effectiveness and Cost-Effectiveness of Community Water Fluoridation

August 2024 – Moore et. al. How effective and cost-effective is water fluoridation for adults and adolescents? The LOTUS 10-year retrospective cohort study

Focus: Assess clinical and cost-effectiveness of water fluoridation for adults and adolescents.

Methods: 10-year retrospective (2010-2020), 6.4 million matched pairs, 12 and older, England

Findings:
- Lifetime reduction in invasive dental procedures of 3%
- Lifetime reduction in cavities of 2%
- Missing teeth no difference
- Reduction of inequities no difference

Conclusion:
- Reduction in invasive dental procedures and cavities
- **Positive ROI but may not be meaningful**

Note: Does **not include the cost of dental fluorosis and lost IQ points**

Reference: Moore et. al. 2024. How effective and cost-effective is water fluoridation for adults and adolescents? The LOTUS 10-year retrospective cohort study.

Slide 23

Summary - Benefits: Economic Value of Water Fluoridation

- Quality of inputs determine the quality of the outputs.
- Even with the decline of benefits from community water fluoridation, literature reports it as a cost-effective intervention.
- Recent cost-effectiveness literature does not include risks of fluoride as a neurotoxin (cost of fluorosis, cost of lost IQ points) from a societal perspective.

Slide 24

Cost: Fluoride as a Neurotoxin

-MAGNITUDE OF IMPACT BETWEEN FLUORIDE & OUTCOMES
-COST OF RISKS: FLUOROSIS
-COST OF RISKS: LOST IQ POINTS

Slide 25

Journal Article Review: Magnitude of Impact

Slide from previous presentation *NTP Monograph Fluoride Tox Review*:

Fluoride & IQ:

- For every 1 mg/L in increased maternal urinary fluoride (MUF) **=> 1.14 – 1.63 IQ points** (Taylor et. al. 2025)
- MUF third trimester Los Angeles CA cohort median 0.8 mg/L and 95 C.I. of 1.89 mg/L (Malin et. al. 2023)
- **~1 IQ point at median and 2-3 IQ points** at 95th C.I. (typical protection level)
- For context - estimated average impact of lead is 2.6 IQ points (McFarland 2022)
- Any contributing shift leftward from a population perspective is of note

Reference(s):
- Taylor et al. Fluoride Exposure and Children's IQ Scores: A Systematic Review and Meta-Analysis. JAMA. 2025 Fluoride Exposure and Children's IQ Scores: A Systematic Review and Meta-Analysis | Pediatrics | JAMA Pediatrics | JAMA Network
- Malin et. al. 2023. Maternal Urinary Fluoride and Child Neurobehavior at Age 36 Months | Public Health | JAMA Network Open | JAMA Network
- McFarland et. al. 2022. Half of US population exposed to adverse lead levels in early childhood - PMC

Slide 26

Cost of Risks

FLUOROSIS

Slide 27

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Perspective: Societal
US$: 2010

Journal Article Review: Cost of Risks

Cost of Fluorosis

December 2014– Ko & Thiessen. A critique of recent economic evaluations of community water fluoridation

Cost of Fluorosis

- In previous studies, not included, and if mentioned, stated as negligible.
- Community water fluoridation, in the absence of other fluoride sources, expected to result in a prevalence of mild-to-very mild (cosmetic) dental fluorosis in about 10% of the population and almost no cases of moderate or severe dental fluorosis.
- In the 1999–2004 NHANES survey, 41% of U.S. children ages 12-15 years were found to have dental fluorosis, including 3.6% with moderate or severe fluorosis.
- National Research Council (NRC) concluded that ''severe dental fluorosis'' qualified as an adverse health effect due to increased risk of caries and loss of dental function.
- Societal costing perspective should include cost of fluorosis.

Reference: Ko & Thiessen. 2014. Full article: A critique of recent economic evaluations of community water fluoridation

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Perspective: Societal
US$: 2010

Journal Article Review: Cost of Risks

Cost of Fluorosis

December 2014– Ko & Thiessen. A critique of recent economic evaluations of community water fluoridation

Estimated Cost

- Each moderate or severe fluorosis tooth receives a porcelain veneer treatment
- Child with fluorosis gets first treatment at age 13.5 years
- Replaced every 12 years
- Cost of veneer $1,000 (lower-end)
- Suggested lifetime cost, one-tooth $2,217, cost driven by Dean's Enamel Fluorosis Index, two-most affected teeth.
- Lifetime cost of veneers for a child with moderate or severe fluorosis $4,434 (low-end)

Estimated Impact

- 3.6% of children 12-15 had moderate or severe fluorosis* but did not provide fluoridation status of children
- Calculated using 5% of children in fluoridated areas have moderate or severe fluorosis
- 1.46% of children at age 13.5 years
- Minimum cost of treating dental fluorosis $4,434 x 5% x 1.46% = **$3.24 PPPY**

Reference: Ko & Thiessen. 2014. Full article: A critique of recent economic evaluations of community water fluoridation
*Beltran-Aguilar et. al. 2010. NCHS Data Brief, Number 53, November 2010

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Perspective: Societal
US$: 2021

Journal Article Review: Cost of Risks

Cost of Fluorosis & Lost IQ Points

November 2025 – Osmunson & Cole. Community Water Fluoridation a Cost-Benefit-Risk Consideration

Focus: Including costs of fluorosis and lost IQ points in community water fluoridation

Methods: Review of literature, propose the addition of and calculate for cost of fluorosis & lost IQ points.

Findings:

- **Benefit:** caries averted, less operational costs $8 to $ 41 PPPY
- **Cost:** fluorosis $126 PPPY
- **Cost:** lower earnings from harm of developmental neurotoxicity $438 PPPY
- **Suggested Net Loss:** $556 PPPY

Conclusion: Fluoridation is not cost-effective if the cost of harm is included

Reference: November 2024. Osmunson & Griffin. Community Water Fluoridation a Cost-Benefit-Risk Consideration
Community Water Fluoridation a Cost–Benefit–Risk Consideration - Osmunson - 2024 - Public Health Challenges - Wiley Online Library

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Perspective: Societal
US$: 2021

Journal Article Review: Cost of Risks

Cost of Fluorosis

November 2025 – Osmunson & Cole. Community Water Fluoridation a Cost-Benefit-Risk Consideration

Assumptions:
- 30% of those on fluoridation will have perceived fluorosis.
- Option A selected by 10%
- Option B selected by 20%

Option A

- Micro-abrasion grinding of the outer layer of enamel, sealants, or resin infiltration (fillings) can improve dental fluorosis appearance and minor functional damage.
- Repeated treatment or "touch up" bleaching and/or minor restorations and re-treatments are estimated every 5 years for a conservative $100 PPPY for 60 years.

Option B

- Highest quality of treatment.
- Comprehensive cosmetic and functional treatments are estimated based on experience and dental insurance fees at $1,200 per tooth.
- Classification of dental fluorosis is based on the two worst teeth, although 1–32 teeth can be damaged.
- An estimate is used here for an average of 10 teeth per person (high-end).
- Treatment is estimated to be replaced an average of four times during a person's life.

Reference: November 2024. Osmunson & Griffin. Community Water Fluoridation a Cost-Benefit-Risk Consideration. Community Water Fluoridation a Cost–Benefit–Risk Consideration - Osmunson - 2024 - Public Health Challenges - Wiley Online Library. 2021 USD

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Perspective: Societal
US$: 2021

Journal Article Review: Cost of Risks

Cost of Risks - Fluorosis

November 2025 – Osmunson & Cole. Community Water Fluoridation a Cost-Benefit-Risk Consideration

Option A

- Clinically based estimated cost of $100 a year per person × 60 years = $6,000.
- Inflation at 3.57% and dental inflation at 4.33% ($1,200) = $7,200
- 20% choose Option A = $1,440
- 1.46% of the population at each age = **$21 PPPY**

Option B

- $1,200 × 10 teeth = $12,000 × 5 treatments = $60,000
- Inflation at 3.57% and dental inflation at 4.33% ($12,000) = $72,000
- 10% choose Option B = $7,200
- 1.46% of the population at each age = **$105 PPPY**

Cost: "Combining Options A of $21 PPPY with Option B of $105 PPPY equals a conservative estimate of **$126 PPPY** for the treatment of dental fluorosis."

Reference: November 2024. Osmunson & Griffin. Community Water Fluoridation a Cost-Benefit-Risk Consideration. Community Water Fluoridation a Cost–Benefit–Risk Consideration - Osmunson - 2024 - Public Health Challenges - Wiley Online Library.

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Cost of Risks

LOST IQ POINTS

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Perspective: Societal
US$: 2021

Journal Article Review: Cost of Risks

Cost of Fluorosis & Lost IQ Points

November 2025– Osmunson & Cole. Community Water Fluoridation a Cost-Benefit-Risk Consideration

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- **Cost:** lower earnings from harm of developmental neurotoxicity $438 PPPY
- **Suggested Net Loss:** $556 PPPY

Conclusion: Fluoridation is not cost-effective if the cost of harm is included

Reference: November 2024. Osmunson & Griffin. Community Water Fluoridation a Cost-Benefit-Risk Consideration
Community Water Fluoridation a Cost–Benefit–Risk Consideration - Osmunson - 2024 - Public Health Challenges - Wiley Online Library

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Perspective: Societal
US$: 2021

Journal Article Review: Cost of Risks

Cost of Risks – Lost IQ Points

November 2025 – Osmunson & Cole. Community Water Fluoridation a Cost-Benefit-Risk Consideration

Estimated Cost

- 1 IQ point increase = $500 increased earnings per year
- Conservative estimate of $500 PPPY

Estimated Impact

- Conservative estimate of 3 IQ points lost
- 3 IQ points x $500/year = $1,500
- Assuming 40 work years x $1,500 = $60,000 lifetime lost wages
- 50% drink a significant amount of CWF = $30,000
- 1.46% of population at each age = $438 PPPY lost wages

Reference: November 2024. Osmunson & Griffin. Community Water Fluoridation a Cost-Benefit-Risk Consideration
Community Water Fluoridation a Cost–Benefit–Risk Consideration - Osmunson - 2024 - Public Health Challenges - Wiley Online Library

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Perspective: Societal
US$: 2024

Journal Article Review: Cost of Risks

2002. Grosse et. al. Economic Gains Resulting from the Reduction in Children's Exposure to Lead in the US.

Table. Converting Estimated cost per IQ point lost in lifetime earnings to 2021 & 2024 US$

Level	Estimated cost per IQ point lost in lifetime earnings	$ Year	Adjusted to 2021 US$	Adjusted to 2024 US$
Lower Bound	$12,700	2000	**$20,349**	$23,036
Base Case	$14,500	2000	**$23,234**	$26,300
Upper Bound	$17,200	2000	**$27,560**	$31,198

Reference: Grosse SD et. al. Economic gains resulting from the reduction in children's exposure to lead in the United States. Environ Health Perspect. 2002. Economic gains resulting from the reduction in children's exposure to lead in the United States – PMC
Adjusted using U.S. BLS CPI Inflation Calculator

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Perspective: Societal
US$: 1999-2010

Journal Article Review: Cost of Risks

Understanding the effects of fluoride as a neurotoxin

February 2021 – Boyle et. al. Estimated IQ points and lifetime earnings lost to early childhood blood lead levels in the United States

Focus: Examined undetermined racial/ethnic disparities in anticipated IQ points and associated lifetime earnings lost to early childhood blood lead.

Methods: Nationally-represented estimates produced using weighted simulation model. Age 2.

Findings:

- Black infants experienced approximately 46–55% greater average estimated loss of grade school IQ points from blood lead than Hispanic or White infants (−1.78 IQ points vs. −1.15 and−1.21 respectively).
- Similar disparities in costs to expected lifetime earnings (−$47,116 USD vs. −$30,393 and − $32,356 respectively).

Conclusion: Black infants experienced higher IQ point and earning loss due to blood lead. Low levels of blood lead explain the majority of estimated lifetime earning loss.

Reference: Boyle et. al. 2021. Estimated IQ points and lifetime earnings lost to early childhood blood lead levels in the United States - ScienceDirect

Slide 37

Summary - Cost: Fluoride as a Neurotoxin

- Literature estimates lost IQ points from fluoride between 1-3
- Societal perspective should include risks of fluoride
  - Literature demonstrates reduction in the historical return on investment when you add the risks
- Learnings from Lead
  - Calculations of lost IQ points should potentially consider:
    - Level of impact from neurotoxin
    - Racial disparities

Slide 38

General Comments & Limitations

- As costs go up, holding everything else constant, less cost effective, lower return on investment.
- Inputs (costs and outcomes) for calculating the cost of risk of community water fluoridation were difficult to verify.
- No sensitivity analysis around estimates, therefore difficult to discern what inputs are driving them.
- Focus was on community water fluoridation; more work should be done investigating literature on costs and outcomes for other modes of fluoride.

Slide 39

Ideal Next Steps

- **Better understand the Impact in Washington.**
  - Prevalence of Excess Fluoridation
- **Conduct cost-effectiveness including other modes of Fluoride.**
- **Conduct Economic Modeling.**
  - Include cost of fluorosis
  - Include cost of lost IQ points
- **Consider the cost of status quo or eliminating water fluoridation.**

Slide 40

Economic Trade-offs

Can we…

- Maximize the benefits of fluoride

while

- Minimizing the risk of fluoride as a neurotoxin

Slide 41

Questions / Discussion

Slide 42

Washington State Department of HEALTH

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May 6; Christine Till; Fluoride and Neurodevelopment

Slide 1

Fluoride and Neurodevelopment

Presentation prepared for the
Washington State Dept. of Health Fluoride Panel

Christine Till, PhD, C.Psych
Faculty of Health, York University, Canada

May 6, 2025

Slide 2

Brief Bio

- PhD (2004) from University of Toronto
  - Neuropsychology with specialization in toxicology and neurosciences
- Licensed Clinical Neuropsychologist
- Parent
- Professor in the Faculty of Health at York University, Toronto, Canada
- I have studied the impact of toxic chemicals (fluoride, solvents, lead, pesticides, and other chemicals) for >25 years
- Research funded by NIH and CIHR
- Research has been published in leading medical and scientific journals
- My fluoride research has been extensively relied upon by environmental and public health agencies and played a major role in the U.S. Federal trial on the safety of community water fluoridation.

2

Slide 3



Slide 4

Disclosure

I have no actual or potential conflict of interest in relation to this program/presentation.

4

Slide 5

Objectives

1. What does the scientific literature demonstrate on the issue of developmental fluoride neurotoxicity?
2. What do we know about potential mechanisms of neurotoxicity?
3. How is the overall evidence on fluoride neurotoxicity relevant to community water fluoridation?

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Slide 6

What is fluoride?

- Naturally occurring mineral
- Fluoride helps to prevent dental decay
- Added to dental hygiene products

- Systemic sources include fluoridated water and water-based beverages/foods
- Many other systemic sources of exposure

Topical



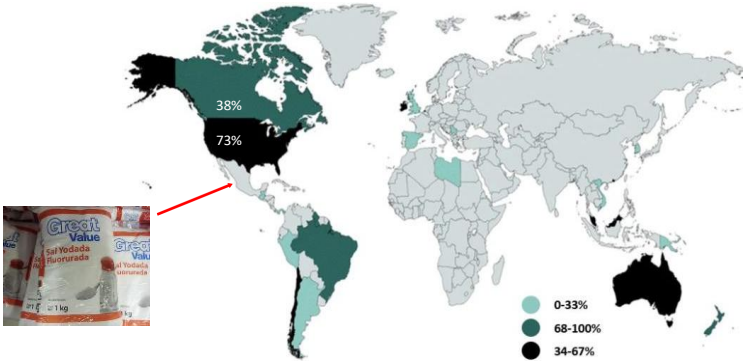
Systemic



6

Slide 7

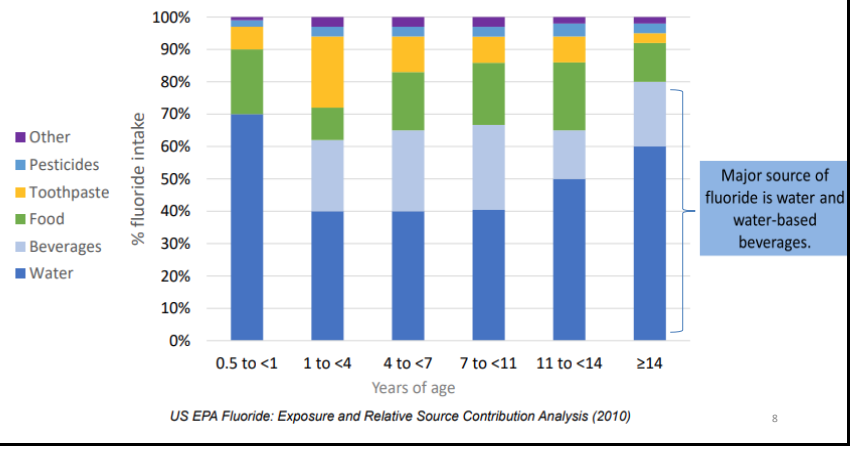
% of population with public water supplemented with fluoride



Johnston & Strobel. *Archives of Toxicology.* (2020)

Slide 8

% of fluoride intake from various sources, across the lifespan



US EPA Fluoride: Exposure and Relative Source Contribution Analysis (2010)

8

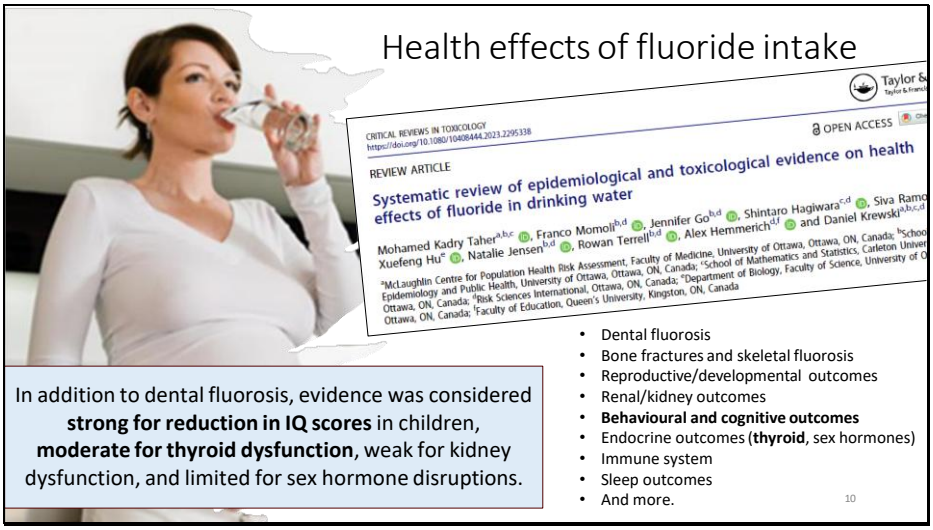
Slide 9

What does the scientific literature demonstrate on the issue of fluoride neurotoxicity?



9

Slide 10



Health effects of fluoride intake

CRITICAL REVIEWS IN TOXICOLOGY
https://doi.org/10.1080/10408444.2023.2295338

OPEN ACCESS

REVIEW ARTICLE

Systematic review of epidemiological and toxicological evidence on health effects of fluoride in drinking water

Mohamed Kadry Taher[a,b,c], Franco Momoli[b,d], Jennifer Go[b,d], Shintaro Hagiwara[c,d], Siva Ramo
Xuefeng Hu[e], Natalie Jensen[b,d], Rowan Terrell[b,d], Alex Hemmerich[d,f] and Daniel Krewski[a,b,c,d]

[a]McLaughlin Centre for Population Health Risk Assessment, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; [b]Schoo
Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada; [c]School of Mathematics and Statistics, Carleton Univer
Ottawa, ON, Canada; [d]Risk Sciences International, Ottawa, ON, Canada; [e]Department of Biology, Faculty of Science, University of O
Ottawa, ON, Canada; [f]Faculty of Education, Queen's University, Kingston, ON, Canada

In addition to dental fluorosis, evidence was considered **strong for reduction in IQ scores** in children, **moderate for thyroid dysfunction**, weak for kidney dysfunction, and limited for sex hormone disruptions.

- Dental fluorosis
- Bone fractures and skeletal fluorosis
- Reproductive/developmental outcomes
- Renal/kidney outcomes
- **Behavioural and cognitive outcomes**
- Endocrine outcomes (**thyroid**, sex hormones)
- Immune system
- Sleep outcomes
- And more.

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Slide 11

Developmental Neurotoxicity of fluoride

- Developing nervous system is especially vulnerable to neurotoxicants
- Some pregnant women and children ingest more fluoride than needed (due to many sources). Must consider total intake.
  - Fluoride stored in bone remobilized into bloodstream during pregnancy
- Fetal exposure
  - Fluoride from maternal blood can readily cross the placenta
  - Penetrates blood-brain barrier
  - Accumulates in brain regions implicated in learning, memory, attention, and executive function



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Slide 12

Developmental Neurotoxicity of fluoride – cont'd

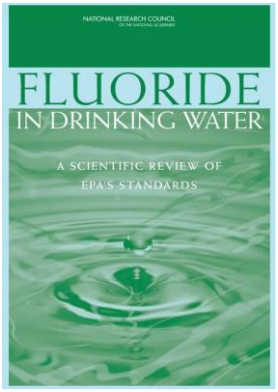
- Formula-fed infants residing in fluoridated communities
  - **3-4x greater exposure** to fluoride than adults on a per body weight basis (NRC, 2006)
  - **70x higher fluoride intake** than exclusively breastfed infants (Zohoori, 2018)
  - Breastmilk contains extremely low levels of fluoride (0.005 mg/L)
- Infants and young children retain 80-90% of absorbed fluoride compared with 50% in adults (Ekstrand, 1994)



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Slide 13

A prior review by the NRC (2006) on the adverse health effects of fluoride concluded:

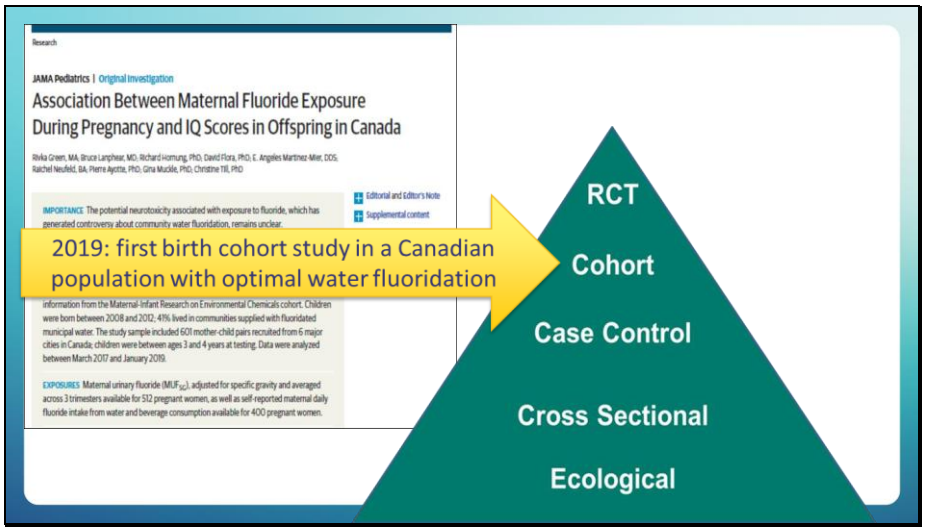


"Further study is necessary, especially for vulnerable populations."

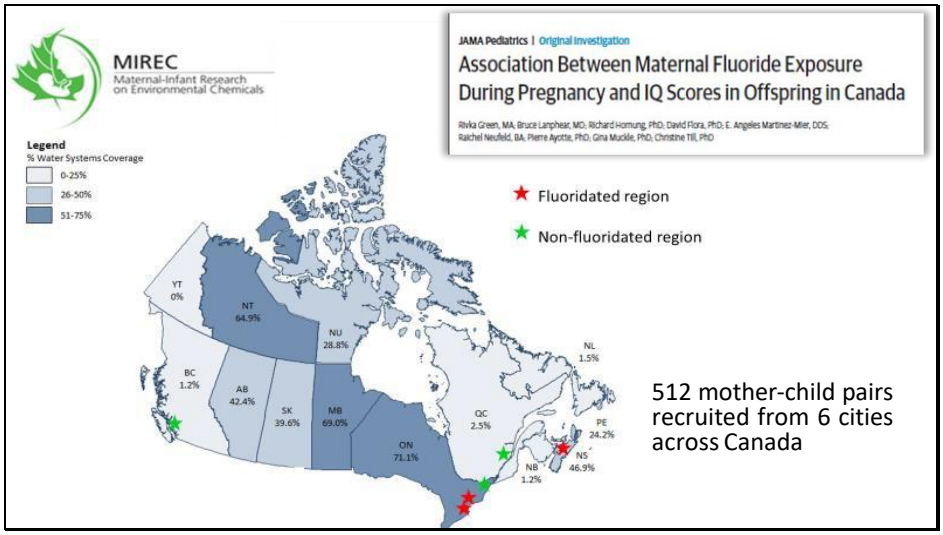
The report "should be a wake-up call".

-Dr. Isaacson, NRC panel member

Slide 14



Slide 15



512 mother-child pairs recruited from 6 cities across Canada

Slide 16

3 measures of fluoride exposure



Maternal urinary fluoride (MUF_{sg}) concentration (mg/L)
*averaged across 3 spot samples

Mean = 0.51 (± 0.36)
RANGE: 0.06-2.44
N = 512



Water fluoride concentration (mg/L)

Mean = 0.32 (± 0.23)
RANGE: 0.04-0.76
N = 420



Fluoride intake (mg F/d)

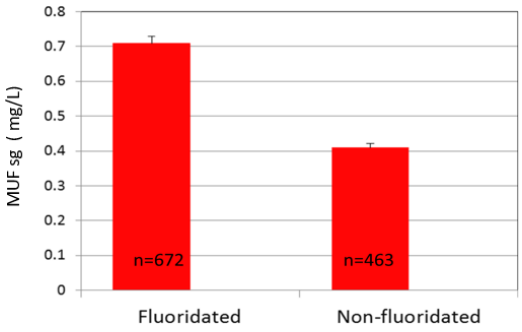
Mean = 0.55 (± 0.46)
RANGE: 0.01-2.65
N = 400

Slide 17



Urinary fluoride levels are 2x higher among pregnant women living in fluoridated vs. non-fluoridated communities

MIREC
Maternal-Infant Research on Environmental Chemicals



Till et al., *Environ Health Perspect*, 2018

Slide 18

Urinary fluoride (mg/L) concentration among pregnant women living in fluoridated communities

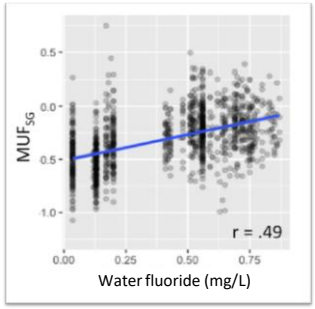
Study	Cohort	Country/ State	N	Median Urinary F (mg/L)	Dilution adjustment
Till et al 2018	**MIREC**	**Canada**	**530** **672**	**0.74** **0.62**	**Creatinine** **SG**
Thomas et al 2016	ELEMENT	Mexico	515	0.91	creatinine
Ibarluzea et al 2022	INMA	Spain	316	0.91	creatinine
Malin et al 2023	MADRES	California	490	0.80	SG
Abduweil et al 2020	--	California	48	0.69	SG
Griebel-Thompson 2025	ADORE	Ohio, Kansas	965	1.0	SG

18

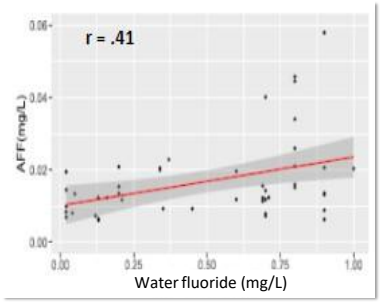
Slide 19



Water fluoride levels are associated with maternal urinary fluoride (MUF) and amniotic fluid fluoride (AFF) levels.



Till et al., *EHP* (2018)



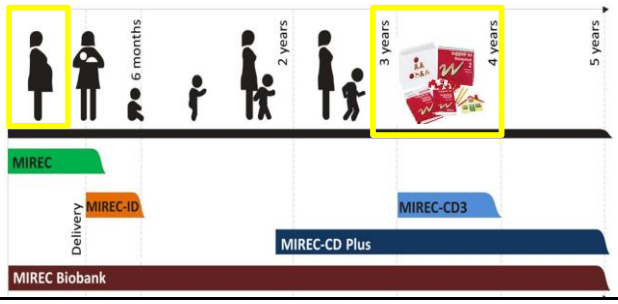
Uyghurturk et al, *Env Health* (2020)

*maternal serum and amniotic fluid: r = .52

Slide 20

MIREC
Maternal-Infant Research on Environmental Chemicals

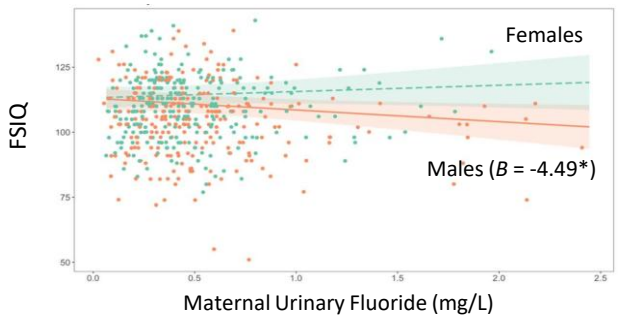
Fluoride-IQ relationships in the MIREC cohort



Slide 21

JAMA Pediatrics | Original Investigation
Association Between Maternal Fluoride Exposure During Pregnancy and IQ Scores in Offspring in Canada

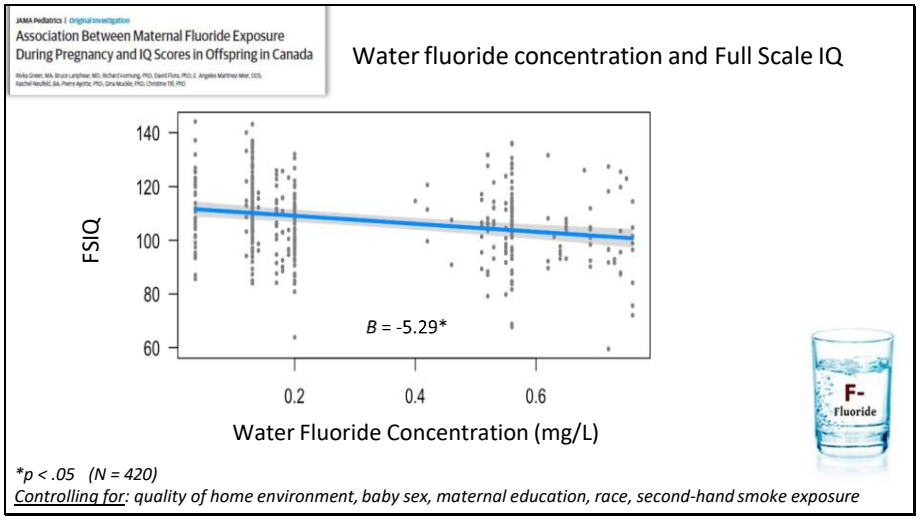
Maternal urinary fluoride and Full Scale IQ



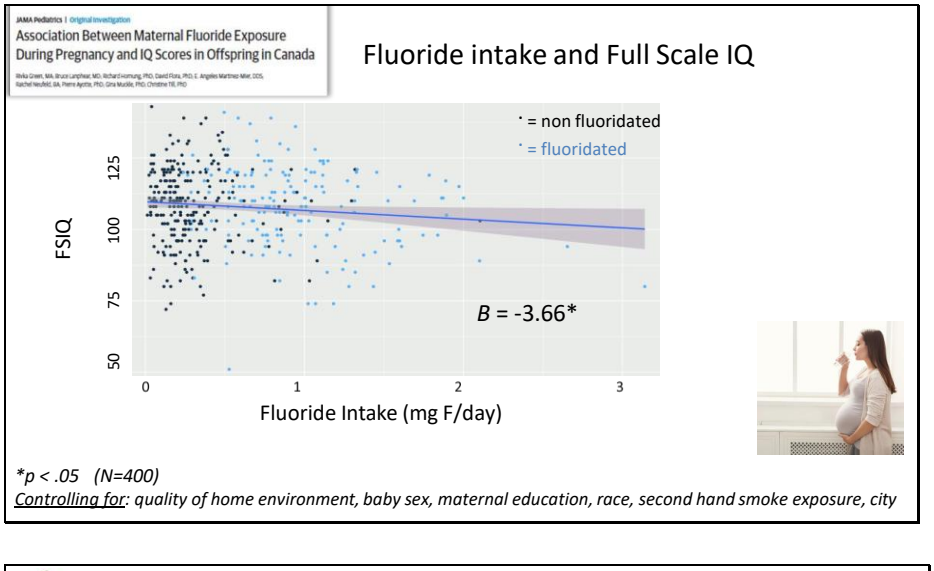


*p < .05 (N=512)
Controlling for: quality of home environment, maternal education, race, and city

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Slide 23



Slide 24

MIREC
Maternal-Infant Research on Environmental Chemicals

Potential confounders considered and/or adjusted for

Child characteristics:
- Sex
- Gestational age
- Weight at birth
- Parity (being first child)
- Age at testing

Paternal characteristics:
- Age
- Education
- Employment status
- Smoking status
- Race/ethnicity

Excluded from study if:
- Known fetal abnormality
- Medical complications
- Illicit drug use during pregnancy

Maternal characteristics:
- City
- Race/ethnicity
- Education
- HOME score
- Exposure to second-hand smoke
- Smoked in trimester 1
- Marital status
- Age at delivery
- Net household income
- Employment status at time of pregnancy
- **Exposure to lead, arsenic, mercury, PFOA, manganese, alcohol**
- Pre-pregnancy body mass index (BMI)
- Chronic condition during pregnancy (e.g. diabetes, depression, high blood pressure)
- Birth country and English as first language
- Parental stress
- Breastfeeding status and duration
- Time of void / time since last void

Slide 25

Sensitivity analyses for MUF-IQ model

MLR Models	*N*	*B (SE)* of MUF among males	*p*	*95% CI*
Model $_A$	512	-4.49 (1.98)	.02	-8.38, -0.60
Model $_{A+lead}$	504	-4.61 (1.98)	.02	-8.50, -0.71
Model $_{A+mercury}$	456	-5.13 (2.05)	.01	-9.16, -1.10
Model $_{A+arsenic}$	512	-4.44 (1.99)	.03	-8.35, 0.54
Model $_{A+second\ hand\ smoke\ exposure}$	512	-4.18 (1.98)	.03	-8.06, -0.30
Model $_{A+prenatal\ alcohol\ consumption}$	512	-4.48 (1.98)	.02	-8.38, -0.59
Model $_B$	510	-4.11 (1.92)	.03	-7.89, -0.33
Model $_C$	407	-4.96 (1.83)	.007	-8.56, -1.36

$Model_A$ – MUF_{SG} controlling for city, HOME score, race and maternal level of education with baby sex as effect modifier

$Model_B$ – $Model_A$ without two FSIQ outliers (males with FSIQ <60)

$Model_C$ – MUF adjusted for creatinine with same covariates as $Model_A$

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One unit increase in fluoride exposure associated with:







UNIT: **1 mg/L** | **1 mg/L** | **1 mg F**

↓ 4.5 IQ pts (*males)

↓ 5.3 IQ pts

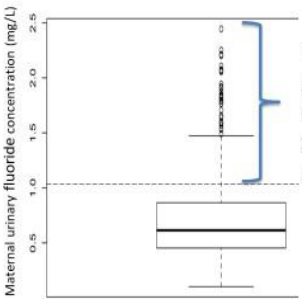
↓ 3.7 IQ pts

Green et al., *JAMA Pediatrics*, 2019

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MIREC
Maternal-Infant Research on Environmental Chemicals

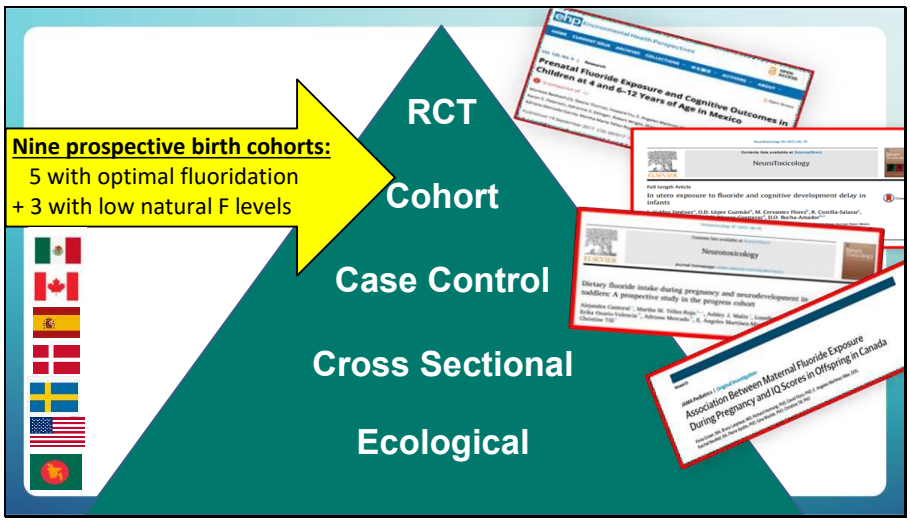
In fluoridated regions, 1 mg/L MUF represents a woman at the 86th percentile. Therefore:



14% of pregnant Canadians have ≥ 1 mg/L urinary fluoride



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Stability of prenatal F-IQ findings

Goodman et al., *Environ Res*, 2022

- F-IQ association **is stable** across ages 4, 5, and 6-12 yrs
- A 0.5 mg/L increase in MUF predicted an average 2.1 decrease in FSIQ/GCI
- Association stronger for Performance IQ than Verbal IQ

ELEMENT

Environ Res. 2022 August ; 211: 112993. doi:10.1016/j.envres.2022.112993.

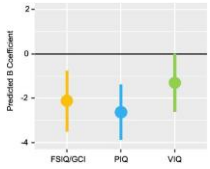
Domain-specific effects of prenatal fluoride exposure on child IQ at 4, 5, and 6–12 years in the ELEMENT cohort

Carly V. Goodman[a], Morteza Bashash[b], Rivka Green[a], Peter Song[c], Karen E. Peterson[c], Lourdes Schnaas[d], Adriana Mercado-Garcia[e], Sandra Martinez-Medina[d], Mauricio Hernández-Avila[f], Angeles Martinez-Mier[g], Martha M. Téllez-Rojo[e,*], Howard Hu[h], Christine Till[a]

Table 2.

MSCA and WASI scores (Mean ± SD) and Pearson correlations among the scores at each time point

			Pearson Correlations		
FSIQ/GCI	N	M ± SD	Age 4	Age 5	Age 6-12
Age 4	386	96.58 ± 13.96	-		
Age 5	308	96.62 ± 12.52	0.76**a	-	
Age 6-12	278	96.20 ± 11.12	0.58**b	0.64**c	-



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Stability of prenatal F-IQ findings

Singh et al., *EHP*, 2025

- 500 mother-child pairs from MINIMat cohort from Bangladesh
- Median MUF = 0.63 mg/L (almost identical to a Californian sample)
- Household water F concentrations ranged from 0.04 to 0.74 mg/L
- A 1 mg/L increase in MUF was associated with a FSIQ raw score decrease of 0.11 to 0.14 standard deviations at ages 5 and 10 years, respectively

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP14534.

Prenatal and Childhood Exposure to Fluoride and Cognitive Development: Findings from the Longitudinal MINIMat Cohort in Rural Bangladesh

Taranbir Singh,[1] Klara Gustin,[1] Syed Moshfiqur Rahman,[2,3] Shamima Shiraji,[3] Fahmida Tofail,[3] Marie Vahter,[1] Mariza Kampouri,[1] and Maria Kippler[1]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[2]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
[3]Maternal and Child Health Division, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

Linear regression of MUF concentration in early pregnancy with child IQ at 5 and 10 years in the MINIMat study

	All children	
Outcomes	*B* (95% CI)	*p*-Value
Cognition at 5 years (WPPSI-III)[b]	*n* = 457	—
Full-scale raw score		
Unadjusted model	−0.2 (−3.1, 2.7)	0.870
Adjusted model	−2.8 (−5.1, −0.6)	0.014
Cognition at 10 years (WISC-IV)[d]	*n* = 500	—
Full-scale raw score		
Unadjusted model	−2.8 (−6.7, 1.1)	0.160
Adjusted model	−4.9 (−8.0, −1.8)	0.002

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Maternal-Infant Research on Environmental Chemicals

What about *postnatal* exposure?





Environment International 134 (2020) 105315

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Fluoride exposure from infant formula and child IQ in a Canadian birth cohort

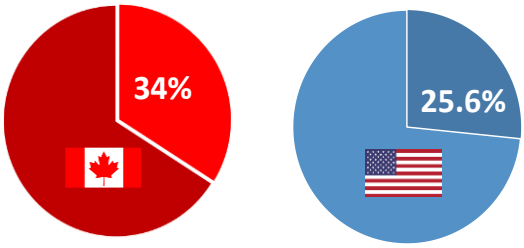
Christine Till[a,*], Rivka Green[a], David Flora[a], Richard Hornung[b], E. Angeles Martinez-Mier[c], Maddy Blazer[a], Linda Farmus[a], Pierre Ayotte[d,e], Gina Muckle[d,f], Bruce Lanphear[g,h]

[a] Faculty of Health, York University, Ontario, Canada
[b] Pediatrics and Environmental Health, Cincinnati Children's Hospital Medical Center, OH, USA
[c] School of Dentistry, Indiana University, IN, USA
[d] Centre de Recherche du CHU de Québec, Université Laval, Québec, Canada
[e] Department of Social and Preventive Medicine, Laval University, Québec, Canada
[f] School of Psychology, Laval University, Québec, Canada
[g] Faculty of Health Sciences, Simon Fraser University, British Columbia, Canada
[h] Child & Family Research Institute, BC Children's Hospital, University of British Columbia, Canada

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Exclusive breastfeeding for the recommended 6 months or more



Therefore, 65-75% of infants receive formula in the first 6 months of life.

Francis et al, IJEH, 2021; Statistics Canada, 2017; CDC, 2020

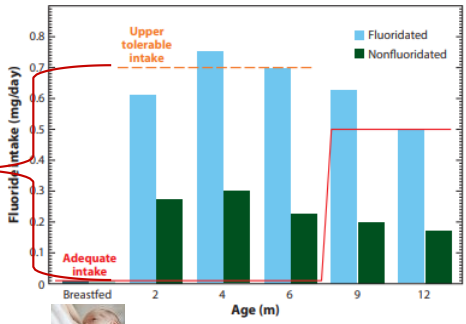
32

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Dietary fluoride intake in infants fed formula reconstituted with fluoridated vs. nonfluoridated water



Fluoride intake is **60-70x** greater among formula-fed infants vs. breastfed infants.



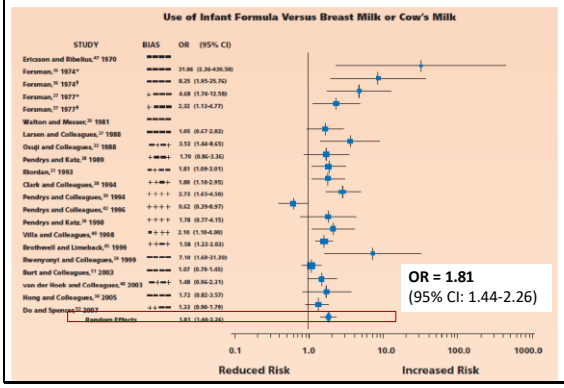


Ekstrand, 1981; Zohoori et al., 2018; Harriehausen et al 2019.

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Risk of enamel fluorosis is higher among formula fed infants.

Hujoel et al., JADA, 2009



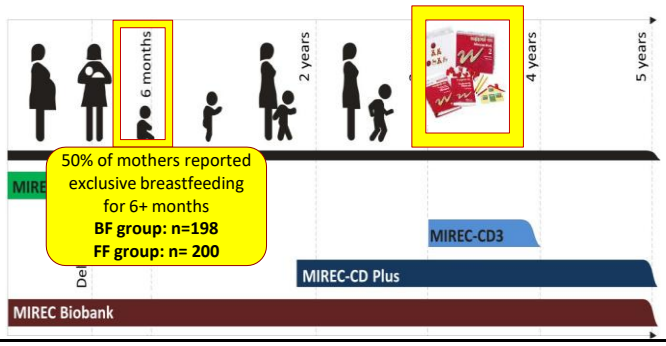


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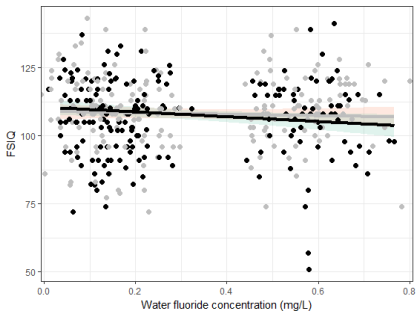
Are formula-fed infants at risk of lower IQ if formula is made with fluoridated water?

Infant feeding → Childhood IQ



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A 0.5 mg/L increase in water fluoride level was associated with a **4.4-point decrement in FSIQ** in the formula-fed (FF) group. No significant association in breastfed (BF) group.





Breastfed: B = -1.34 (-5.04, 2.38)
Formula: B = -4.40 (-8.34, -0.46)*



Till et al., Envir Intern, 2020

*Controlling for child sex, age, maternal educ, race, smoke in home, HOME

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Are there any benefits of using fluoridated tap water to mix infant formula?



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No.

Fluoride's predominant beneficial effect is post-tooth eruption!

(Berg et al, 2011; Limeback, 1999; Featherstone, 2001; NRC, 2006; Warren & Levy, 2003)

American Academy of Pediatrics recommends fluoride tablets only after primary teeth erupt

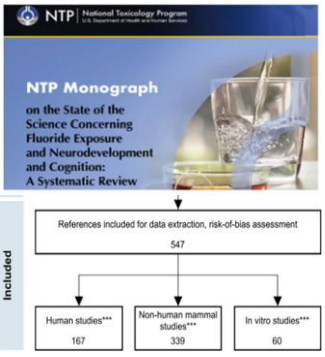
- Only if child is susceptible to high caries activity and not exposed to other fluoride-based interventions

Table. DIETARY FLUORIDE SUPPLEMENTATION SCHEDULE

Age	<0.3 ppm F	0.3 to 0.6 ppm F	>0.6 ppm F
Birth to 6 months	0	0	0
6 mo to 3 years	0.25 mg	0	0
3 to 6 years	0.50 mg	0.25 mg	0
6 to at least 16 years	1.00 mg	0.50 mg	0

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A more comprehensive evaluation of the developmental neurotoxicity of fluoride




Research

JAMA Pediatrics | Original Investigation

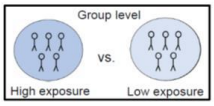
Fluoride Exposure and Children's IQ Scores
A Systematic Review and Meta-Analysis

Kyla W. Taylor, PhD; Sorina E. Eftim, PhD; Christopher A. Sibrizzi, MPH; Robyn B. Blain, PhD; Kristen Magnuson, MESM; Pamela A. Hartman, MEM; Andrew A. Rooney, PhD; John R. Bucher, PhD

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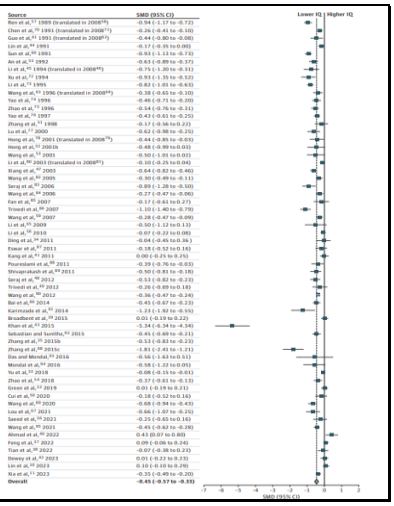
National Toxicology Program

Group Level Meta-analysis #1:



"52 of 59 (88%) studies reported an inverse association between fluoride exposure and children's IQ."

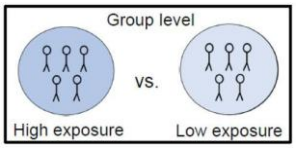
Pooled standardized mean effect difference (SMD): **-0.45** (-0.57, -0.33) (~6.75 IQ points)

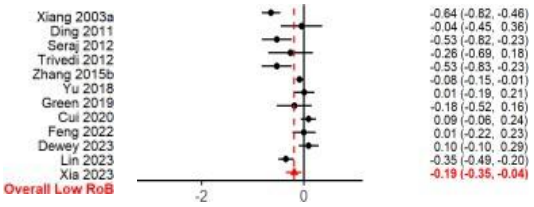


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National Toxicology Program

Consistent association if only include higher quality studies (*n* = 12).

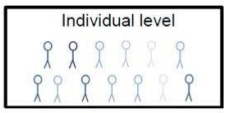




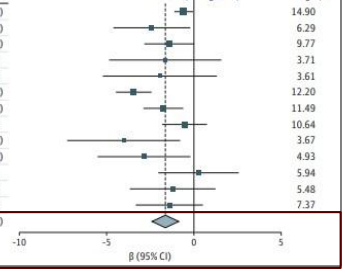
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National Toxicology Program

Regression Slopes Meta-Analysis #2:



Source	Exposure	β (95% CI)	Weight, %
Ding et al,[34] 2011	Per 1 mg/L urinary fluoride	-0.59 (-1.09 to -0.09)	14.90
Zhang et al,[35] 2015	Per 1 mg/L urinary fluoride	-2.42 (-4.59 to -0.25)	6.29
Cui et al,[99] 2018	Per 1 mg/L urinary fluoride	-1.41 (-2.81 to -0.01)	9.77
Yu et al,[33] 2018	Per 1 mg/L urinary fluoride	-1.65 (-4.83 to 1.53)	3.71
Green et al,[32] 2019	Per 1 mg/L maternal urinary fluoride	-1.95 (-5.18 to 1.28)	3.61
Saeed et al,[36] 2021	Per 1 mg/L urinary fluoride	-3.45 (-4.44 to -2.46)	12.20
Zhao et al,[100] 2021	Per 1 mg/L urinary fluoride	-1.76 (-2.86 to -0.65)	11.49
Feng et al,[37] 2022	Per 1 mg/L urinary fluoride	-0.54 (-1.79 to 0.71)	10.64
Goodman et al,[101] 2022	Per 1 mg/L maternal urinary fluoride	-4.02 (-7.22 to -0.82)	3.67
Tian et al,[38] 2022	Per 1 mg/L urinary fluoride	-2.86 (-5.48 to -0.24)	4.93
Grandjean et al,[9] 2023	Per 1 mg/L maternal urinary fluoride	0.26 (-2.02 to 2.54)	5.94
Lin et al,[10] 2023	Per 1 mg/L urinary fluoride	-1.22 (-3.64 to 1.21)	5.48
Xia et al,[11] 2023	Per 1 mg/L urinary fluoride	-1.40 (-3.29 to 0.50)	7.37
Overall		-1.63 (-2.33 to -0.93)	



Results show an inverse association between urinary fluoride exposure and child IQ.

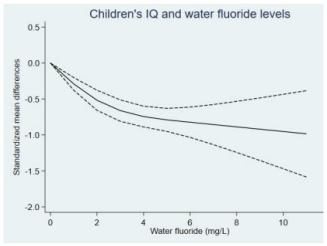
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National Toxicology Program

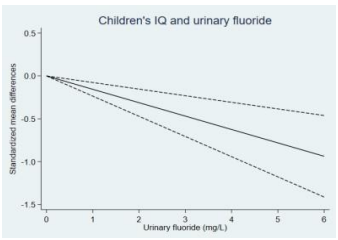
Dose-Response Mean Effects Meta-Analysis #3:

IQ drops with increasing levels of F in water and urine.

There is no obvious threshold.



N= 31 studies with water F exposure and at least two exposure levels.



N = 20 studies with urinary F exposure and at least two exposure levels.

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Dose-Response Mean Effects Meta-Analysis #3:



Equivalent to an IQ decrement of 1.2 points

Taylor et al., 2025

Table 2. Pooled Changes in Standardized Mean Differences (SMDs) From the Linear Model From the Dose-Response Mean-Effects Meta-Analyses Using Group-Level Measures of Fluoride Exposure

Fluoride exposure, mg/L	Studies, No.	Effect estimates, No.[a]	Children, No.	Parameter estimates[b] β (95% CI)	*P* value
Urinary fluoride, all studies					
All data	20	32	9756	−0.15 (−0.23 to −0.07)	<.001
<4	14	25	8019	−0.20 (−0.31 to −0.08)	.001
<2	6	10	4692	−0.08 (−0.15 to −0.005)	.04
<1.5	5	8	4219	−0.08 (−0.15 to −0.003)	.04
Urinary fluoride, low risk-of-bias studies					
All data	10	14	6847	−0.13 (−0.23 to −0.03)	.01
<4	10	14	6847	−0.13 (−0.23 to −0.03)	.01
<2	4	7	4179	−0.08 (−0.15 to −0.002)	.04
<1.5	4	7	4179	−0.08 (−0.15 to −0.002)	.04

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Dose-Response Mean Effects Meta-Analysis #3:



Water F levels <2 mg/L associated with an IQ decrement of ~5 points

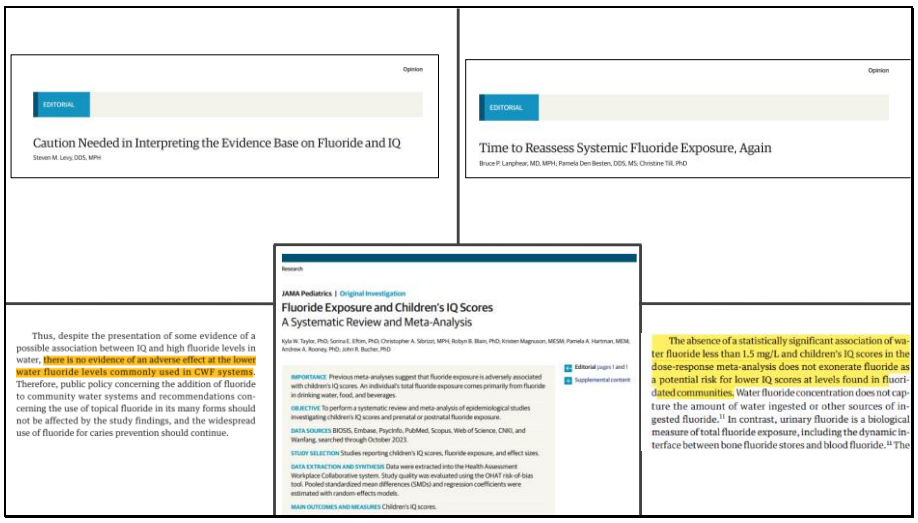
Coefficient remains the same, but no longer significant at <1.5 mg/L

Taylor et al., 2025

Table 2. Pooled Changes in Standardized Mean Differences (SMDs) From the Linear Model From the Dose-Response Mean-Effects Meta-Analyses Using Group-Level Measures of Fluoride Exposure

Fluoride exposure, mg/L	Studies, No.	Effect estimates, No.[a]	Children, No.	Parameter estimates[b] β (95% CI)	*P* value
Water fluoride, all studies					
All data	31	41	12 487	−0.15 (−0.20 to −0.11)	<.001
<4	23	29	9554	−0.22 (−0.27 to −0.17)	<.001
<2	8	10	3682	−0.18 (−0.40 to 0.03)	.10
<1.5	7	7	2832	0.05 (−0.36 to 0.45)	.82
Water fluoride, low risk-of-bias studies					
All data	7	12	5066	−0.21 (−0.33 to −0.09)	.001
<4	7	10	4962	−0.23 (−0.34 to −0.11)	<.001
<2	4	5	1632	−0.33 (−0.53 to −0.13)	.001
<1.5	3	3	879	−0.32 (−0.91 to 0.26)	.28
Urinary fluoride, all studies					
All data	20	32	9756	−0.15 (−0.23 to −0.07)	<.001
<4	14	25	8019	−0.20 (−0.31 to −0.08)	.001
<2	6	10	4692	−0.08 (−0.15 to −0.005)	.04
<1.5	5	8	4219	−0.08 (−0.15 to −0.003)	.04
Urinary fluoride, low risk-of-bias studies					
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<2	4	7	4179	−0.08 (−0.15 to −0.002)	.04
<1.5	4	7	4179	−0.08 (−0.15 to −0.002)	.04

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Exposure Considerations



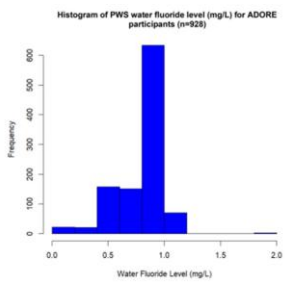


- Provides useful estimate of long-term population exposure
- Underestimates total exposure because it does not capture amount of water ingested or other sources of fluoride.

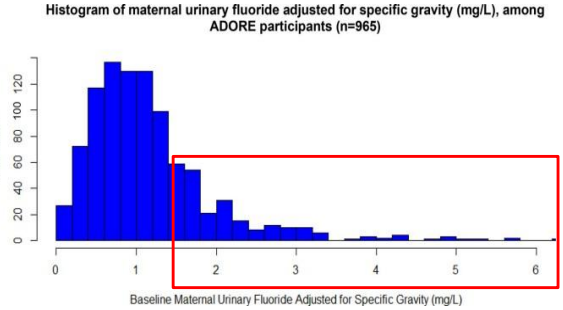
- Urinary fluoride is a reliable biological measure that captures an individual's <u>total</u> fluoride exposure
- Represents a limited (recent) time-period
- Influenced by when sample was taken and differences in dilution
- Routinely used by regulatory agencies for risk assessment

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Evidence of fluoride neurotoxicity at urine F levels <1.5 mg/L is relevant to community water fluoridation.



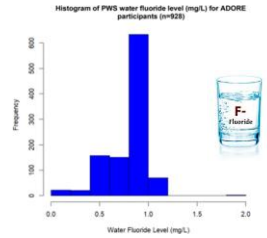






Griebel-Thompson et al., EHP, 2025

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Pregnant women (and children) can exceed 1.5 mg/L depending on amount of fluoridated water ingested and exposure to other sources.

Table 2. Median (Q1, Q3) baseline and third trimester maternal urinary fluoride levels (adjusted for specific gravity) among ADORE participants and estimated fluoride intake by PWS water fluoridation level.

	p	All cohort (*n* = 965)	Water fluoridation[a] <0.7 mg/L (*n* = 215) [median (IQR)]	Water fluoridation[a] ≥0.7 mg/L (*n* = 713) [median (IQR)]	*p*-Value[b]	Missing (*n*)
Baseline maternal urinary fluoride, adjusted (mg/L)[c]	965	1.0 (0.6, 1.5)	0.8 (0.5, 1.2)	1.0 (0.7, 1.5)	<0.01	—
Third trimester maternal urinary fluoride, adjusted (mg/L)[c]	307	1.1 (0.8, 1.6)	1.1 (0.8, 1.4)	1.1 (0.8, 1.6)	0.28	658
Fluoride intake (mg)[d]	728	0.4 (0.1, 1.4)	0.2 (0.1, 0.8)	0.4 (0.1, 1.9)	<0.01	237

Note: —, no data; ADORE, Assessment of DHA on Reducing Early Preterm Birth; IQR, interquartile range; PWS, public water systems.
[a]PWS water fluoridation level missing for *n* = 37 participants.
[b]Mann-Whitney U tests were used to determine significance.
[c]Adjusted for specific gravity. Baseline specific gravity [median (Q1, Q3)], 1.0 (1.0, 1.0); third trimester specific gravity [median (Q1, Q3)], 1.0 (1.0, 1.0), missing 658.
[d]Fluoride intake was calculated by multiplying the participant's PWS water fluoridation level by self-reported amount of tap water consumed (daily average).

Griebel-Thompson et al., EHP, 2025

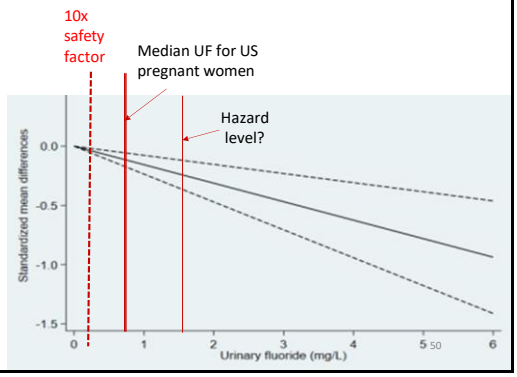
Slide 50

Based on the evidence of fluoride neurotoxicity at 1.5 mg/L, fluoridation at 0.7 mg/L offers a safety margin of 2x.

"Under even the most conservative estimates, there is not enough of a margin between the accepted hazard level (i.e., 1.5 mg/L) and the actual human exposure levels to find that fluoride is safe."

Judge Chen, October 2024





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MIREC
Maternal-Infant Research on Environmental Chemicals

Iodine deficiency may increase risk of fluoride neurotoxicity.

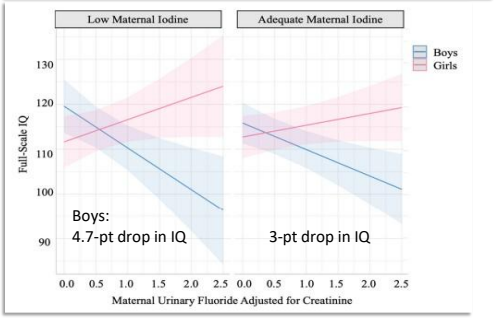
Goodman et al., *Nutrients*, 2022;

nutrients

Article

Iodine Status Modifies the Association between Fluoride Exposure in Pregnancy and Preschool Boys' Intelligence

Carly V. Goodman [1], Meaghan Hall [1], Rivka Green [1], Jonathan Chevrier [2], Pierre Ayotte [3], Esperanza Angeles Martinez-Mier [4], Taylor McGuckin [1], John Krzeczkowski [1], David Flora [1], Richard Hornung [5,†], Bruce Lanphear [6] and Christine Till [1,*]



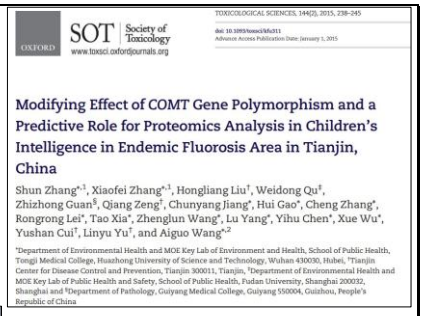
*3-way interaction significant for boys (p = .04), but not girls (p =.19)

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Genetic factors

Individuals with certain genetic variants in dopamine receptor D2 or the catechol-O-methyltransferase (*COMT*) gene may have heightened sensitivities to fluoride exposure (Cui et al., 2018; Zhang et al., 2015).



SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 144(2), 2015, 238–245

Modifying Effect of COMT Gene Polymorphism and a Predictive Role for Proteomics Analysis in Children's Intelligence in Endemic Fluorosis Area in Tianjin, China

Shun Zhang*,1, Xiaofei Zhang*,1, Hongliang Liu†, Weidong Qu‡, Zhizhong Guan§, Qiang Zeng†, Chunyang Jiang*, Hui Gao*, Cheng Zhang*, Rongrong Lei*, Tao Xia*, Zhenglun Wang*, Lu Yang*, Yihu Chen*, Xue Wu*, Yushan Cui†, Linyu Yu†, and Aiguo Wang*,2

*Department of Environmental Health and MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, †Tianjin Center for Disease Control and Prevention, Tianjin 300011, Tianjin, ‡Department of Environmental Health and MOE Key Lab of Public Health and Safety, School of Public Health, Fudan University, Shanghai 200032, Shanghai and §Department of Pathology, Guiyang Medical College, Guiyang 550004, Guizhou, People's Republic of China



Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Dopamine receptor D2 gene polymorphism, urine fluoride, and intelligence impairment of children in China: A school-based cross-sectional study

Yushan Cui[a,1], Bin Zhang[b,1], Jing Ma[c], Yang Wang[c], Liang Zhao[c], Changchun Hou[a], Jingwen Yu[b], Yang Zhao[c], Zushan Zhang[c], Junyan Nie[b], Tongning Gao[b], Guoli Zhou[b,c], Hongliang Liu[b,d,e,*]

[a] Tianjin Centers for Disease Control and Prevention, 6 Huayue Road, Hedong District,Tianjin 300011, PR China
[b] School of Public Health, Tianjin Medical University, 22 Qixiangtai Road, Heping District, Tianjin 300070, PR China
[c] Tianjin Cardiovascular Institute, Tianjin Chest Hospital, 261 Taierzhuang south road, Jinnan District, Tianjin 300050, PR China
[d] Biomedical Research Informatics Core (BRIC), Clinical and Translational Sciences Institute (CTSI), Michigan State University, 909 Fee Road, East Lansing, MI 48824,

Genotype	*N*	IQ points lost per 1 mg/L urine F	*p*-value
combined	108	–2.42	0.030
val/val	28	–9.67	0.003

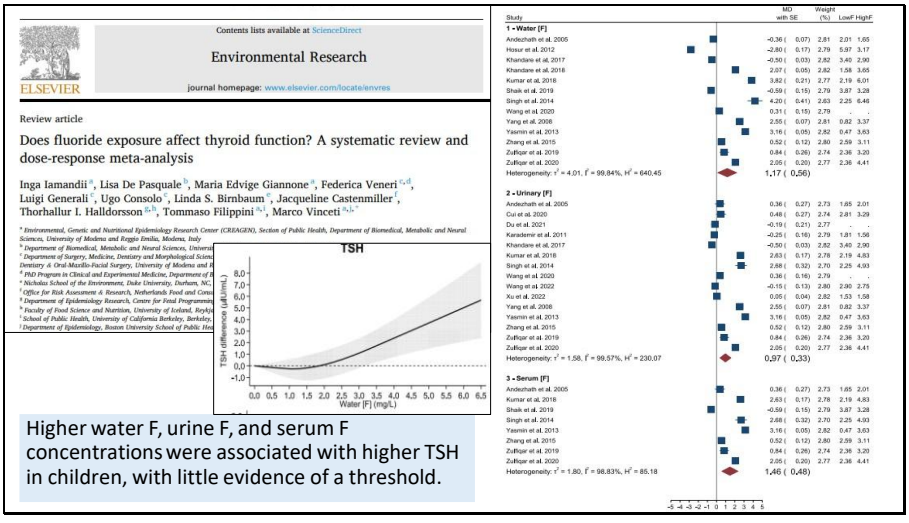
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Mechanisms of Fluoride Neurotoxicity

- Contribute to mitochondrial dysfunction
- Increase oxidative stress
- Alter cholinergic activity
  - α4 and α7 nAChRs subunits
- Alter glutamate metabolism
- Decreases in neural receptors and stunted neuronal development
- *Thyroid hormone disruption

Barbier et al. 2010. Molecular mechanisms of fluoride toxicity. Chem. Biol. Interact. 188(2):319–33
Johnston et al., 2020. Principles of fluoride toxicity and the cellular response: a review. Arch. Toxicol. 94(4):1051–69

Slide 54



Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

Does fluoride exposure affect thyroid function? A systematic review and dose-response meta-analysis

Inga Iamandii[a], Lisa De Pasquale[b], Maria Edvige Giannone[a], Federica Veneri[c,d], Luigi Generali[e], Ugo Consolo[e], Linda S. Birnbaum[f], Jacqueline Castenmiller[f], Thorhallur I. Halldorsson[g,h], Tommaso Filippini[a,i], Marco Vinceti[a,i,*]

[a] Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[b] Department of Biomedical, Metabolic and Neural Sciences, Universi
[c] Department of Surgery, Medicine, Dentistry and Morphological Scienc
Dentistry & Oral-Maxillo-Facial Surgery, University of Modena and R
[d] PhD Program in Clinical and Experimental Medicine, Department of M
[e] Nicholas School of the Environment, Duke University, Durham, NC,
[f] Office for Risk Assessment & Research, Netherlands Food and Cons
[g] Department of Epidemiology Research, Centre for Fetal Programmin
[h] Faculty of Food Science and Nutrition, University of Iceland, Reykja
[i] School of Public Health, University of California Berkeley, Berkeley,
[j] Department of Epidemiology, Boston University School of Public Hea

Higher water F, urine F, and serum F concentrations were associated with higher TSH in children, with little evidence of a threshold.

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Contents lists available at ScienceDirect

Science of the Total Environment 2023

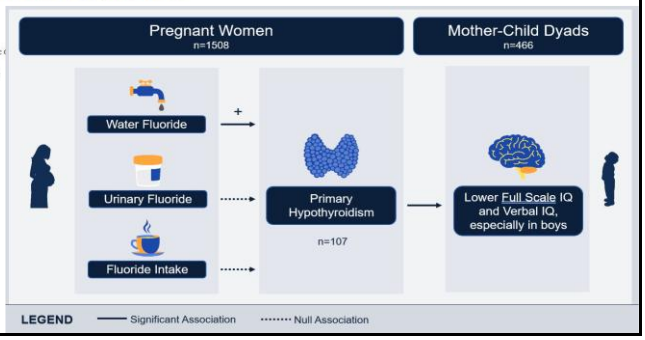
journal homepage: www.elsevier.com/locate/scitotenv

MIREC Maternal-Infant Research on Environmental Chemicals

Fluoride exposure and hypothyroidism in a Canadian pregnancy cohort

Meaghan Hall [a], Bruce Lanphear [b], Jonathan Chevrier [c], Rick Hornung [d], Rivka Green [a], Carly Goodman [a], Pierre Ayotte [e], Esperanza Angeles Martinez-Mier [f], R. Thomas Zoeller [g], Christine Till [a,*]

[a] Psychology Department, York University, Toronto, ON, Canada
[b] Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada
[c] School of Population and Global Health, McGill University, Montreal, QC, Canada
[d] Retired; Consultant to Psychology Department, York University, Toronto, ON, Canada
[e] Department of Social and Preventive Medicine, Faculty of Medicine, Université Laval, Québec
[f] School of Dentistry, Indiana University, Indianapolis, IN, United States
[g] Biology Department, The University of Massachusetts Amherst, Amherst, MA, United States

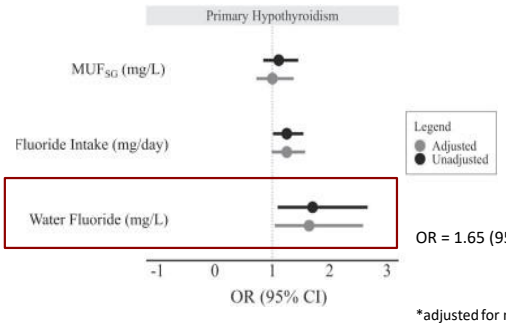


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MIREC Maternal-Infant Research on Environmental Chemicals



Pregnant women (N =1105) exposed to higher concentrations of fluoride in drinking water were at higher risk of hypothyroidism.



OR = 1.65 (95% CI: 1.04, 2.60)

*adjusted for maternal age, pre-pregnancy BMI, maternal education, race, and city of residence
*also: Tg, As, Pb, Mg, Hg, PFAS

Hall et al., *STOTEN*, 2023

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MIREC
Maternal-Infant Research on Environmental Chemicals

Published in final edited form as:
Sci Total Environ. 2024 July 10; 933: 173121. doi:10.1016/j.scitotenv.2024.173121.

Letter to the editor regarding Hall et al. (2023): Fluoride exposure and hypothyroidism in a Canadian pregnancy cohort

Meaghan Hall[a], Bruce Lanphear[b], Jonathan Chevrier[c], Rick Hornung[d], Rivka Green[a], Carly Goodman[a], Pierre Ayotte[e], Esperanza Angeles Martinez-Mier[f], R. Thomas Zoeller[g], Christine Till[a]

Association between water fluoride concentration and risk of primary hypothyroidism.

	n	aOR*	95% CI	*p*
Total Sample	1105	1.65	1.04, 2.60	.03
+ Lived at residence for ≥ 1 year	889	1.80	1.07, 3.01	.03
+ Normal[a] TPO Ab Levels	1094	2.85	1.25, 6.60	.01

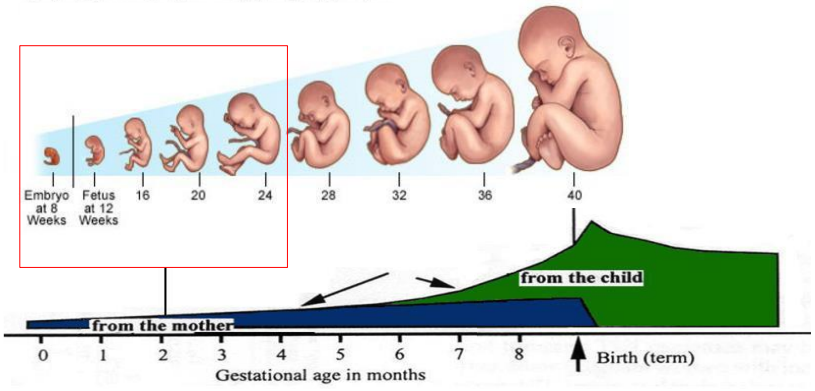
[a] TPO Ab < 5.61 IU/mL.
* adjusted OR reported for 0.5 mg/L increase in water fluoride concentration.

Hall et al. (*2023*). *STOTEN.*

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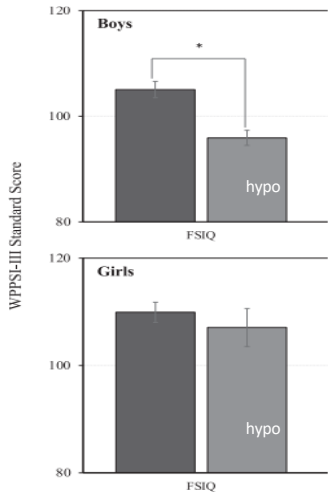
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Thyroid hormone is critical in gestational development



Thorpe-Beeston & Nicholaides, 1995

Slide 59



MIREC
Maternal-Infant Research on Environmental Chemicals



Children whose mothers had hypothyroidism (n=28) had **lower IQ scores** than children whose mothers had normal thyroid levels (n=411), especially for boys.

Hall et al., *STOTEN*, 2023

Slide 60



Research

JAMA Pediatrics | Original Investigation

Fluoride Exposure and Children's IQ Scores
A Systematic Review and Meta-Analysis

Kyla W. Taylor, PhD; Sorina E. Eftim, PhD; Christopher A. Sibrizzi, MPH; Robyn B. Blain, PhD; Kristen Magnuson, MESM; Pamela A. Hartman, MEM; Andrew A. Rooney, PhD; John R. Bucher, PhD

"Confidence in the associations at lower fluoride levels could be increased by additional prospective cohort studies with individual fluoride exposure measures."

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7 of 9 prospective birth cohort studies report adverse effects of gestational exposure to fluoride.

Prospective Birth Cohort	Significant adverse effects (lower IQ, behavior problems) reported	No adverse effects reported
Optimal fluoridation	**ELEMENT**: Bashash 2017/Goodman 2022 (Mexico) **MIREC**: Green 2019/Till 2020 (Canada) **PROGRESS**: Cantoral 2021 (Mexico) **MADRES***: Malin 2024 (USA)	**INMA**: Ibarluzea 2022 (Spain)
Low natural F	**NICE***: Kampouri 2025 (Sweden) **MINIMat**: Singh 2025 (Bangladesh)	**OCC**: Grandjean 2023 (Denmark)
High natural F	Valdez-Jiminez 2017 (Mexico)	

*MUF associated with increased risk of behavioral problems

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8 of 10 prospective birth cohort studies report adverse effects of gestational exposure to fluoride.

Prospective Birth Cohort	Significant adverse effects (lower IQ, behavior problems) reported	No adverse effects reported
Optimal fluoridation	**ELEMENT**: Bashash 2017/Goodman 2022 (Mexico) **MIREC**: Green 2019/Till 2020 (Canada) **PROGRESS**: Cantoral 2021 (Mexico) **MADRES***: Malin 2024 (USA)	**INMA**: Ibarluzea 2022 (Spain)
Low natural F	**NICE***: Kampouri 2025 (Sweden) **MINIMat**: Singh 2025 (Bangladesh) + ***NHBCS****: *in progress (USA)*	**OCC**: Grandjean 2023 (Denmark)
High natural F	Valdez-Jiminez 2017 (Mexico)	

*MUF associated with increased risk of behavioral problems

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Fluoride and Neurobehavioural Outcomes

Ecologic Studies:

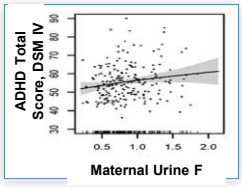
- USA: Higher rates of ADHD found in states with most CWF compared to those with least (Malin & Till, *Envir Health*, 2015)
- Canada: Higher tap water F associated with poorer inhibitory control (Dewey et al., 2023)

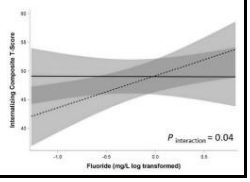
Prenatal F Studies:

- Mexico: Higher maternal urinary F associated with more ADHD-like symptoms in children (Bashash et al., *EHP*, 2017)
- USA: Higher maternal urinary F associated with increased risk of internalizing problems and somatic complaints (Malin et al, JAMA Network, 2024)
- Sweden: Higher maternal urinary F associated with ADHD problems in children (Kampouri et al., *Env Res*, 2024)

Cross sectional studies:

- Canada: Higher tap water F concentration associated with higher prevalence of ADHD (Riddell et al., *Environ Int'l*, 2019)
- USA: Higher child urine F associated with increased somatization and internalizing symptoms in males (Adkins et al., 2021)

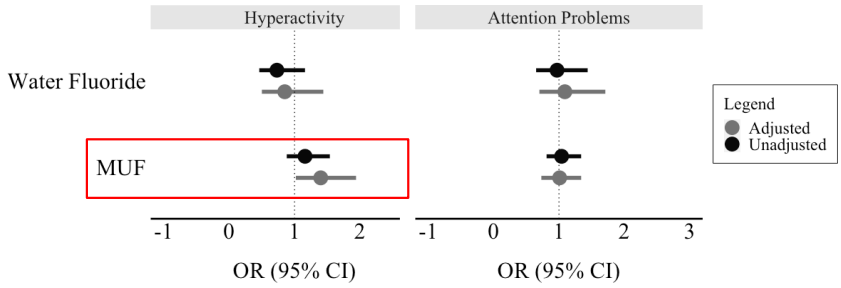




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MIREC
Maternal-Infant Research on Environmental Chemicals

Higher maternal urinary F level in pregnancy associated with elevated symptoms of hyperactivity. (OR=1.42; 95%CI (1.03-1.95)



Radmilovic et al., in preparation

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Criteria for Causality (Bradford Hill, 1965)









- Strength of the association
- Consistency
- Temporality
- Biological plausibility
- Dose-response relationship
- Coherence
- Experimental evidence

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Why should we care about a few IQ points?



Shift of 3 IQ points*

*3 IQ points selected based on Grandjean et al's (2022) benchmark dose model showing that a urinary F level of 0.28 mg/L is associated with a loss of 1 IQ point. 3 IQ points is a rough estimate if we factor in both prenatal and postnatal exposure and consider total fluoride intake.

Little Things Matter, Lanphear

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ANNUAL REVIEWS

Annual Review of Public Health

Health Risks and Benefits of Fluoride Exposure During Pregnancy and Infancy

Christine Till,[1] Philippe Grandjean,[2,3] E. Angeles Martinez-Mier,[4] Howard Hu,[5] and Bruce Lanphear[6]

[1]Faculty of Health, York University, Toronto, Ontario, Canada; email: ctill@yorku.ca
[2]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island, USA
[3]Department of Public Health, University of Southern Denmark, Odense, Denmark
[4]Department of Dental Public Health and Dental Informatics, School of Dentistry, Indiana University, Indianapolis, Indiana, USA
[5]Keck School of Medicine, University of Southern California, Los Angeles, California, USA
[6]Faculty of Health Sciences, Simon Fraser University, Vancouver, British Columbia, Canada

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Given that fluoride offers little benefit to the fetus and young infant, community-wide administration of systemic fluoride may pose an unfavorable risk–benefit ratio for the pregnant woman, fetus, and infant.

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Acknowledgements

Co-Investigators
- Dr. Bruce Lanphear
- Dr. David Flora
- Dr. Rick Hornung
- Dr. Gina Muckle
- Dr. Pierre Ayotte

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- Rivka Green
- Meaghan Hall
- Carly Goodman
- Linda Farmus
- Ana Radmilovic
- Raichel Neufeld
- Dr. John Krzeczkowski
- Dr. John Grundy

MIREC
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- Dr. Tye Arbuckle
- Nicole Lupien
- Romy McMaster

INSPQ Staff
- Alain LeBlanc and staff

Indiana University
- Dr. Angeles Martinez-Mier
- Dr. Frank Lippert
- Sharon Gwinn

Funding

NIH National Institutes of Health

CIHR IRSC Canadian Institutes of Health Research

WEPGN Water Economics, Policy and Governance Network

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Thank you

ctill@yorku.ca



Slide 70

IQ scores are valid in MIREC study

1. FSIQ is an aggregate of different cognitive skills (e.g. verbal/nonverbal reasoning). Considered the **most valid and reliable** measure of overall cognitive ability. Proven validity for use with diverse samples and across the lifespan.
2. Psychometrists underwent **rigorous training** to ensure competency in test administration.
   - Completed a 3-day training session that was led by a PhD-level psychologist
   - Integrity of test administration ensured by conducting **regular site visits** to observe testers.
3. Test protocols **double scored** by a PhD level supervisor to ensure accuracy of scores and consistency in how responses are interpreted across sites.
4. Regression models **controlled for study site**, which would control for variability in test administration between cities.
   - Results remained consistent (and significant)
5. Removed data from 9 children (1.5% of sample who underwent IQ testing) for whom the IQ data were not deemed valid or did not complete the test in its entirety.

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Why developmental neurotoxicology studies measure IQ in preschool-aged children:

- Environmental factors (e.g. neurotoxic exposures) are responsible for a larger proportion of the variance than genetics among younger-aged children.
- The longer the time following toxic exposure, the more opportunities there are for other environmental factors (e.g. education, home envir) to impact IQ

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Stability of IQ scores

Intelligence 69 (2018) 8–18

Contents lists available at ScienceDirect

Intelligence

journal homepage: www.elsevier.com/locate/intell

Stability of intelligence from infancy through adolescence: An autoregressive latent variable model

Huihui Yu[a,*], D. Betsy McCoach[b], Allen W. Gottfried[c], Adele Eskeles Gottfried[d]

[a] Yale University, United States
[b] University of Connecticut, United States
[c] Fullerton Longitudinal Study, California State University, Fullerton, United States
[d] California State University, Northridge, United States

Table 2
Correlation matrix of IQ scores.

	Measures	Age (in years)	1	2	3	4	5	6	7	8	9	10	11	12
1.	*BSID* MDI	1.0	–											
2.		1.5	0.41**	–										
3.		2.0	0.43**	0.62**	–									
4.	*MSCA* GCI	2.5	0.33**	0.63**	0.64**	–								
5.		3.0	0.37**	0.65**	0.67**	0.79**	–							
6.		3.5	0.37**	0.54**	0.68**	0.74**	0.76**	–						
7.	*WISC-R*	6.0	0.26**	0.45**	0.60**	0.57**	0.59**	0.67**	–					
8.		7.0	0.22*	0.41**	0.55**	0.56**	0.59**	0.63**	0.79**	–				
9.		8.0	0.20*	0.42**	0.54**	0.55**	0.59**	0.62**	0.79**	0.83**	–			
10.		12.0	*0.17*	0.39**	0.51**	0.42**	0.47**	0.47**	0.72**	0.78**	0.80**	–		
11.	*WISC-III*	15.0	*0.15*	0.35**	0.48**	0.40**	0.45**	0.45**	0.64**	0.70**	0.77**	0.80**	–	
12.	WAIS-R	17.0	*0.16*	0.39**	0.43**	0.44**	0.49**	0.44**	0.67**	0.70**	0.77**	0.82**	0.85**	–

Note. BSID MDI = Bayley Scales of Infant Development Mental Development Index; MSCA GCI = McCarthy Scales of Children's Abilities General Cognitive Index; WISC-R = Wechsler Intelligence Scales for Children-Revised; WISC-III = Wechsler Intelligence Scale for Children Third Edition; WAIS-R = Wechsler Adult Intelligence Scale-Revised. Statistically non-significant correlations are in italics and underlining.

* $p < .05$.
** $p < .01$.

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Benchmark concentration value
(i.e. urine F concentration that corresponds to a 1-point IQ loss)

Derived using 3 cohort studies:
MIREC, ELEMENT, OCC (n=1599)

European Journal of Public Health, 1–7
Published by Oxford University Press on behalf of the European Public Health Association 2023.
This work is written by (a) US Government employee(s) and is in the public domain in the US.
https://doi.org/10.1093/eurpub/ckad170

Dose dependence of prenatal fluoride exposure associations with cognitive performance at school age in three prospective studies

Philippe Grandjean [1,2], Alessandra Meddis [3], Flemming Nielsen [1], Iben H. Beck [1], Niels Bilenberg[4], Carly V. Goodman[5], Howard Hu[6], Christine Till[7], Esben Budtz-Jørgensen[3]

Table S3. BMC values for creatinine-adjusted maternal U-F (mg/L) for three models and for both sexes of the three studies and the combined data, where the outcome in the ELEMENT study is the McCarthy GCI score. The fit of the regression models was compared by the AIC (where lower values indicate a better fit).

		OCC (n= 837)		MIREC (n=407)		ELEMENT GCI (n=355)		All three studies (n=1,599)		
	Sex	BMC	BMCL	BMC	BMCL	BMC	BMCL	BMC	BMCL	AIC
Linear	All	0.920	0.303	0.497	0.228	0.245	0.142	0.474	0.284	12552
	Girls	0.487	0.189	∞	0.609	0.182	0.106	0.633	0.280	12550
	Boys	3.609	0.309	0.201	0.125	0.426	0.149	0.393	0.225	12550

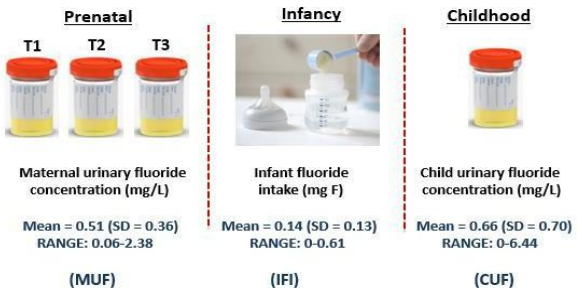
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MIREC
Maternal-Infant Research on Environmental Chemicals

Contents lists available at ScienceDirect
Environmental Research
journal homepage: www.elsevier.com/locate/envres
ELSEVIER

Critical windows of fluoride neurotoxicity in Canadian children

Linda Farmus[a], Christine Till[b,*], Rivka Green[a], Richard Hornung[b], E. Angeles Martinez Mier[c], Pierre Ayotte[d,e], Gina Muckle[d,f], Bruce P. Lanphear[b,g], David B. Flora[a]



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MIREC
Maternal-Infant Research on Environmental Chemicals

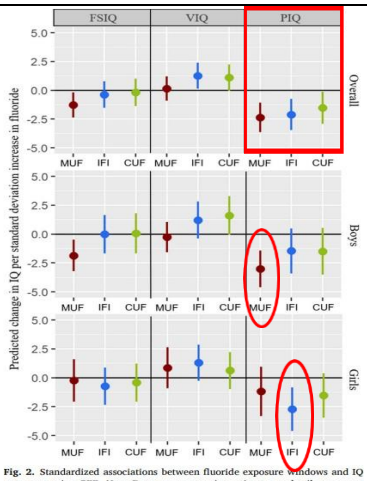


Fig. 2. Standardized associations between fluoride exposure windows and IQ outcomes using GEE. *Note*. Dots represent point estimates and tails represent 95% confidence intervals.

For overall sample, both the prenatal and infancy periods were critical.

For boys, prenatal period was critical.

For girls, infancy period was critical.

Farmus et al., 2021

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Animal studies on fluoride and sex effects



- Mullenix et al. (1995):
  - male rats most sensitive to fluoride in the late prenatal period, female rats most sensitive in the postnatal period
- Findings are consistent with some (e.g. Baran-Poesine et al., 2013; Bera et al., 2007; Flace et al., 2010), but not all (e.g. Bartos et al., 2015; Jiang et al., 2014) rat studies examining sex-specific effects of prenatal exposure to fluoride
- Further research should examine sex-specific effects of fluoride neurotoxicity as many of the animal studies conducted to date have been identified as having a high risk of bias (NTP, 2016)

Reviewed in Green et al., 2020

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What does the current scientific literature indicate about the benefits of community water fluoridation?



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October 2024

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Cochrane Database of Systematic Reviews

[Intervention Review]

Water fluoridation for the prevention of dental caries

Zipporah Iheozor-Ejiofor[1], Tanya Walsh[2], Sharon R Lewis[2], Philip Riley[2], Dwayne Boyers[3], Janet E Clarkson[2,4], Helen V Worthington[2], Anne-Marie Glenny[2], Lucy O'Malley[2]

[1]School of Medicine, University of Central Lancashire, Preston, UK. [2]Cochrane Oral Health, Division of Dentistry, School of Medical Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK. [3]University of Aberdeen, Aberdeen, UK. [4]Division of Oral Health Sciences, School of Dentistry, University of Dundee, Dundee, UK

Older evidence may not be applicable to contemporary societies where fluoride toothpastes and other preventative measures are widely used.



Reviewed studies conducted post-1975:
1. To evaluate the effects of CWF for the prevention of dental caries (dmft/DMFT; n=21);
2. To evaluate the association of CWF with dental fluorosis (n=90)



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Cochrane Database of Systematic Reviews

[Intervention Review]

Water fluoridation for the prevention of dental caries

Zipporah Iheozor-Ejiofor[1], Tanya Walsh[2], Sharon R Lewis[2], Philip Riley[2], Dwayne Boyers[3], Janet E Clarkson[2,4], Helen V Worthington[2], Anne-Marie Glenny[2], Lucy O'Malley[2]

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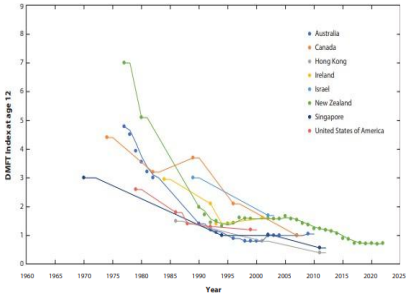
Main conclusions:

- There is a **much smaller benefit of CWF** compared with pre-1975 studies.
- CWF led to a reduction in number of decayed, missing, and filled primary teeth (dmft) of **not more than 4% or possibly no benefit** given uncertainty of the estimate.
- **Fluorosis at 0.7 mg/L**: 12% of aesthetic concern; 40% had fluorosis of any level.
- **Inconsistent evidence** to show that CWF reduces oral health inequalities
- **Insufficient evidence** to determine the effect of cessation of CWF on caries.

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Dental caries trends over last 50 years

Fluoridated countries

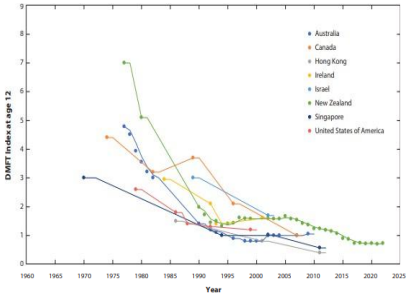


Figures based on Country/Area Profile Program data accessed from the WHO Collaborating Centre for Education, Training, and Research in Oral Health, Malmö University, Sweden.

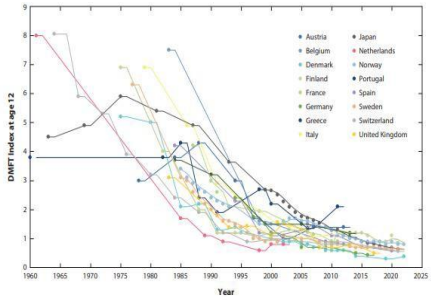
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Dental caries trends over last 50 years

Fluoridated countries



Non-fluoridated countries



Figures based on Country/Area Profile Program data accessed from the WHO Collaborating Centre for Education, Training, and Research in Oral Health, Malmö University, Sweden.

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PERSPECTIVE

Water fluoridation for the prevention of dental caries

Cochrane Oral Health

Lucy O'Malley, Jan Clarkson, Sharon Lewis, Tanya Walsh and Anne-Marie Glenny are oral health researchers based at the Universities of Manchester and Dundee. They are all authors on the 'Cochrane review of water fluoridation' and members/Coordinating Editors/Editors for *Cochrane Oral Health*.

Key points

- Early studies of community water fluoridation showed an important effect on prevention of tooth decay in children. Recent studies show smaller effects.
- Any potential oral health benefits should be comprehensively considered alongside potential harms and costs (taking contemporary effect estimates into account).
- There is inconsistent evidence with regards to the impact of water fluoridation on oral health inequalities.
- Where community water fluoridation is implemented, monitoring of water fluoride concentrations is critical to ensure schemes consistently provide optimal potential benefits in terms of caries prevention.

no conclusive evidence for an association between optimal CWF and most conditions evaluated.[3,5]

Given that observed benefits have reduced over time, before introducing new CWF schemes, careful thought needs to be given to costs and ability to properly implement

> 'We must be open to changes in the evidence, even when they challenge strongly held beliefs.'

O'Malley et al (2025). British Dental Journa, 238 (4): 241-42.

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Research Reports: Clinical

Water Fluoridation and Dental Caries in U.S. Children and Adolescents

Journal of Dental Research
2018, Vol. 97(10) 1122–1128
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sagepub.com/journalsPermissions.nav
DOI: 10.1177/0022034518774331
journals.sagepub.com/home/jdr

G.D. Slade[1], W.B. Grider[2], W.R. Maas[3], and A.E. Sanders[1]

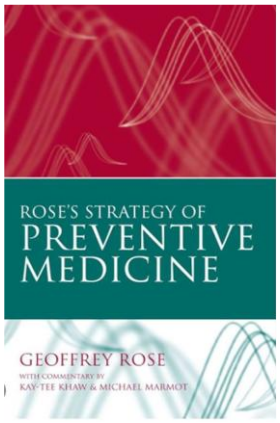
Table 4. Unadjusted Mean Caries Experience According to Fluoridation Status among U.S. 2- to 17-y-olds: National Health and Nutrition Examination Survey 1999 to 2004 and 2011 to 2014.

	2- to 8-y-old dfs		6- to 17-y-old DMFS	
% of County Fluoridated[a]	*n*	Mean (95% CL)	*n*	Mean (95% CL)
<75% of county population (a)	2,914	4.6 (3.9, 5.4)	5,107	2.2 (2.0, 2.4)
≥75% of county population (b)	4,086	3.3 (2.8, 3.7)	7,497	1.9 (1.8, 2.1)
Absolute difference: (a) minus (b)		1.3 (0.6, 2.2)		0.3 (0.0, 0.5)
Prevented fraction: [(a) minus (b)]/(a) × 100 (%)		30 (11, 48)		12 (1, 23)

CL, confidence limit; dfs, number of decayed or filled primary tooth surfaces per person; DMFS, number of decayed, missing, or filled permanent tooth surfaces per person; *n*, unweighted number of participants in descriptive sample.
[a]Percentage of county population served by public water systems with ≥0.7 ppm fluoride.

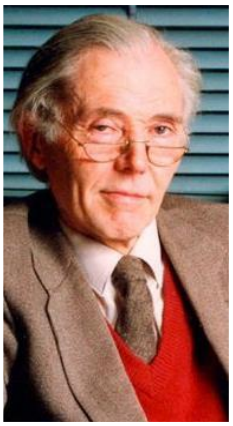
Conclusion:
Findings show a significant caries-preventive benefit of CWF for U.S. children. The benefit is most pronounced in primary teeth.

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"People can buy toothpaste with or without added fluoride, but if fluoride is added to the drinking water, they can hardly avoid imbibing it.
We should expect a higher level of scientific evidence and popular acceptability for measures such as water fluoridation which are imposed and not chosen by the recipients."

Geoffrey Rose, pp. 148



WASHINGTON STATE BOARD OF HEALTH

Working for a safer and healthier Washington since 1889

Recommended Strategies to Improve the Oral Health of Washington Residents

Goals:

- To promote strategies which are consistent with *Healthy People 2020* in order to improve the oral health of Washington residents
- To reduce oral health disparities among Washington residents
- To guide Washington State Board of Health (SBOH) rule and policy development activity
- To provide leadership on public health policies that focus on oral health promotion, prevention, early intervention, and treatment

The following strategic recommendations are based on a review of established evidence and best practice models, consultation with expert informants, input from Washington state and National expert oral health review panels. The recommendations are not intended to be a comprehensive list of available strategies, but should be considered by communities, organizations, and agencies seeking to promote oral health in the State of Washington. Special consideration was given to oral health strategies that are evidence based, cost effective, and impact high risk populations. These seven important strategies taken together will significantly improve the oral health of Washington residents.

State Board of Health Strategic Recommendations

Health Systems: Support policies and programs that improve oral health for Washington state residents.

- Maintain and build on effective programs, like Access to Baby and Child Dentistry, University of Washington Regional Initiatives in Dental Education (RIDE), and adult Medicaid coverage
- Examine cost-effective measures to strengthen Washington's dental public health infrastructure
- Explore cost containment measures to reduce inefficient oral health costs – for example decrease unnecessary emergency room use for dental issues
- Evaluate incentives for healthcare providers who provide services to low income adults and special populations, including diabetics and pregnant women
- Support dedicated staffing to lead a statewide oral health coalition and measure the impact of oral health programs

Community Water Fluoridation: Expand and maintain access to community water fluoridation for the health benefit of children, adults, and seniors.

- Support communities that currently provide optimal levels of fluoride to their residents and those seeking to adopt community water fluoridation.
- Support efforts to educate and inform Washington state residents about the importance of fluoridation to improve community health.

- Engage with organizations, agencies and coalitions to promote community water fluoridation in Washington state

Sealant Programs: Provide school-age children with access to dental sealants to prevent cavities.

- Promote school based sealant programs aligned with the Centers for Disease Control's expert work group recommendations for school-based sealant programs

Interprofessional Collaboration: Incorporate oral health improvement strategies across healthcare professions (such as medicine, nursing, social work, and pharmacy) and systems to improve oral health knowledge and patient care.

- Encourage the State of Washington's healthcare systems and providers to incorporate oral health into their practices
- Encourage health focused educational institutions to incorporate and maintain oral health in their curricula
- Explore innovative collaborative approaches to improve interprofessional delivery of oral health services - for example explore oral health models used by other states
- Support strategies that focus on high risk groups like pregnant women, children, seniors, and those with exacerbating chronic conditions like diabetes or HIV/AIDS

Oral Health Literacy: Improve the capacity of people to obtain, understand, and use health information in order to increase their acceptance and adoption of effective oral health focused preventive practices.

- Encourage collaboration to provide consistent and culturally relevant oral health messaging in settings with at-risk populations: perinatal, senior centers, and early learning (such as Head Start, child care, and home visiting programs; and Women, Infants, and Children Food and Nutrition Services)
- Collaborate with diverse organizations to promote oral health - for example, engage with the Office of Drinking Water, community based anti-obesity efforts, and private enterprise in order to promote healthy behaviors like drinking water, healthy eating habits, reducing tobacco use, and preventing mouth injuries

Surveillance: Monitor trends in oral health indicators to ensure policies and programs are advancing the oral health of Washington residents, including those most at risk for poor oral health outcomes.

- Maintain the Washington State Smile Survey to monitor the oral health of preschool, kindergarten, and elementary school-age children; and the Washington State Oral Disease Burden Document to monitor the oral health of all residents
- Implement oral health surveillance systems for vulnerable populations, including patients enrolled in Medicaid or State Children's Health Insurance Program, homeless, and elders.
- Utilize surveillance tools, including BRFSS, PRAMS, and Cancer Registry among others, to design and track measurable goals and objectives toward improving oral health among Washington residents

Work Force: Develop health professional policies and programs which better serve the dental needs of underserved populations.

- Develop programs to mentor, recruit and train students of color in the dental professions.
- Investigate options to serve rural and underserved communities - for example expanding the University of Washington Dental RIDE program and increasing the number of community health centers
- Research the best ways to recruit and develop a workforce to provide care for the dental underserved regions in our state - for example partnerships with academic institutions, and new strategies to recruit and retain dental professionals
- Support policies for the exploration and feasibility of new and emerging evidence based dental workforce models to increase access to and efficiency of dental treatment.

Fluoridation of Public Water Systems: Federal, State, and Local Roles

This fact sheet was developed by the State Board of Health policy staff as an educational resource for Board Members. It is intended solely for informational purposes and should not be considered or relied upon as legal advice.

Background:
Fluoride is a mineral that occurs naturally in the environment, including in soil, rocks, and water. Most drinking water sources contain some level of fluoride due to natural processes such as the breakdown of rocks and minerals.[1,2] Fluoride may also be added to everyday products like toothpaste and to public drinking water in a controlled way to help prevent tooth decay.[3]

Community Water Fluoridation (CWF) is the practice of adjusting fluoride levels in public water systems to help reduce cavities.[4] While some water systems may have naturally occurring fluoride, other systems choose to add it.[5] Approximately 64% of the population in Washington State drinks water from a public water system that fluoridates.[6]

Decision-Making Authority for CWF:

- In the U.S., the decision to fluoridate public water systems is made at the state or local level.[7,8]
- The federal government provides direction and guidance on the safety and effectiveness of CWF.
    - The U.S. Environmental Protection Agency (EPA) sets national safety standards for fluoride in drinking water (4.0 mg/L and 2.0 mg/L) under the Safe Drinking Water Act of 1974 (SDWA).
    - The U.S. Public Health Service (USPHS) recommends the optimal fluoride level in public water systems for systems that choose to fluoridate (currently 0.7 mg/L).
- In Washington State, decisions about whether to fluoridate public water systems are made at the local level, by public water systems or local governments.[5]
- RCW 70.05.060 (3) authorizes local boards of health to enact rules necessary to preserve and improve public health, including whether to require fluoridation of public water systems within their jurisdiction (AGO 2008 No. 5).
- In addition, RCW 57.08.012 authorizes fluoridation of a water district's water by a majority vote of water district commissioners.
- If communities choose to fluoridate, they must follow public water system rules set by the Washington State Board of Health (Board).

State Board of Health's (Board) Role:

- State law (RCW 43.20.050) requires the Board to set health and safety standards for public water systems to ensure safe drinking water.
- Under this regulatory authority, the Board:
    - Sets the *optimal fluoride level* for large public water systems (Group A) that choose to fluoridate. This is established in state rule (WAC 246-290-460) and is currently aligned with the U.S. Public Health Service (USPHS) recommendation of 0.7 mg/L (last updated in 2015).

    - Adopts the maximum contaminant level (MCL) for fluoride in drinking water (WAC 246-290-310), consistent with federal standards. This determines the highest allowable concentration of fluoride before a water system must take corrective action. The MCL adopted by the Board must be at least as stringent as the MCL established in the SDWA.
- These are two distinct regulatory authorities:
    - The *optimal fluoride level* supports public health benefits for systems that choose to fluoridate.
    - The *MCL* addresses potential health risks from fluoride, whether from natural sources or added sources that exceed the optimal level.
- The optimal level is based on guidance from USPHS, while the MCL is based on standards set by the U.S. Environmental Protection Agency (EPA).
- The Board does not require or ban fluoridation in its rules.

Federal Government's Role:

- The federal government's role in regulating water fluoridation is limited. [7]
- The two agencies primarily involved in CWF are: the Environmental Protection Agency (EPA) and the United States Public Health Service (USPHS).
- The federal government does not require communities to add or remove fluoride from drinking water, as long as levels stay below the EPA's Maximum Contaminant Level (MCL) of 4.0 mg/L.
- USPHS does not have the same public health authority as state or local governments to regulate water fluoridation. They only issue guidance. This guidance is not a regulation, and it is not legally binding.

Federal Agencies and their Roles:

U.S. Environmental Protection Agency (EPA)

- Regulates fluoride levels in public drinking water under the 1974 Safe Drinking Water Act (SDWA).[9]
- Sets both primary (enforceable) and secondary (non-enforceable) standards for fluoride in drinking water.[7]
- The primary standard sets the maximum contaminant level (MCL) at 4.0 mg/L to protect against health risks.[10]
- The secondary standard sets the MCL at 2.0 mg/L as a guideline, primarily to address cosmetic concerns such as tooth discoloration.[11]
- Focuses on managing fluoride concentrations that pose health risks, whether naturally occurring or added and exceeding the optimal level.[7]

U.S. Public Health Service (USPHS)

- Part of the U.S. Department of Health and Human Services (HHS), USPHS includes the Centers for Disease Control (CDC), Food and Drug Administration (FDA), and Agency for Toxic Substances and Disease Registry (ATSDR).
- Recommends the *optimal fluoride level* in public water systems that choose to fluoridate (currently 0.7 mg/L) to prevent tooth decay while minimizing health risks.

- Provides *guidance and recommendations* to support state and local decision-making on fluoridation. These are not enforceable standards.[4,7]

U.S. Food and Drug Administration (FDA)

- Also under HHS – oversees the safety and effectiveness of fluoride in consumer products such as toothpaste, mouthwash, and dietary supplements.[12] This authority is granted through the Food, Drug, and Cosmetic Act (FD&C Act).[13]
- Sets labeling and safety standards for fluoride-containing products sold to the public.
- Coordinates with the CDC, EPA, and USDA to ensure product safety and consumer protection under the FD&C Act.
- Regulates fluoride as an over-the-counter drug when it is intended to prevent or treat disease. In the case of fluoride, the disease is tooth decay.[14,15]
- Regulates fluoride levels in bottled water and ensures that those standards are compatible with EPA regulations for public drinking water. When the EPA sets a new standard for a contaminant, the FDA either adopts the same standard for bottled water or determines it is not necessary.[16]
- Regulates fluoride levels in bottled water but does not regulate fluoride in public drinking water—that role belongs to the EPA under the SDWA.

U.S. Department of Agriculture (USDA)

- Oversees farming, food safety, and national nutrition programs.[17]
- Collects and shares data on fluoride levels in food and beverages.[18,19]
- Provides dietary guidance through tools like the National Nutrient Database and the Dietary Reference Intakes (DRIs), which consider fluoride intake from food sources.
- Agency does *not* regulate fluoride in drinking water or consumer products.

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[15] Food and Drug Administration. Is It a Cosmetic, a Drug, or Both? (Or Is It Soap?). FDA. September 11, 2024. Accessed July 22, 2025. https://www.fda.gov/cosmetics/cosmetics-laws-regulations/it-cosmetic-drug-or-both-or-it-soap

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Fluoridation of Public Water Systems: Federal, State, and Local Roles

Fluoride is a mineral found naturally in the environment, including in soil, rocks, and water. It can also be added to everyday products like toothpaste, mouthwash, and drinking water. Fluoride is commonly used to help prevent cavities and tooth decay.

Community Water Fluoridation is a public health practice used in many communities across the U.S. It involves adjusting the amount of fluoride in public drinking water to a level that helps reduce tooth decay. Some water systems naturally have fluoride; others may choose to add it.

Level of Government	Agency/Entity	Role in Water Fluoridation
Federal	U.S. Environmental Protection Agency (EPA)	Sets enforceable maximum contaminant levels (MCLs) for fluoride under the Safe Drinking Water Act; regulates public water systems for safety.
	U.S. Public Health Services (USPHS)	Recommends the optimal fluoride level (currently 0.7 mg/L); provides non-binding guidance for states and local governments on water fluoridation to promote oral health.
	U.S. Food and Drug Administration (FDA)	Regulates fluoride in consumer products (toothpaste, supplements, bottled water); coordinates with other agencies to ensure product safety and consumer protection.
	U.S. Department of Agriculture (USDA)	Tracks fluoride levels in food and beverages; provides dietary intake guidance.
State	Washington State Board of Health (Board)	Sets health and safety standards for Group A public water systems in Washington under chapter 246-290 WAC; establishes the optimal fluoride level for systems that practice water fluoridation; adopts federal MCL standards. The Board does not mandate or prevent community water fluoridation.
	Washington State Department of Health (Department)	Enforces the rules established in Chapter 246-290 WAC; conducted the Fluoride Science Review Panel to review the emerging science and provide recommendations to the Board for review.
Local	Public Water Systems / Local Governments	Make the final decision on whether to fluoridate water; implement fluoridation policies following state standards if chosen.

Understanding Community Water Fluoridation (CWF): State and Federal Roles - Frequently Asked Questions

This document was prepared by the State Board of Health policy staff as a plain-language educational resource for Board Members and the general public. It is provided for informational purposes only and does not constitute legal advice.

What is fluoride?

Fluoride is a mineral found naturally in the environment, including in soil, rocks, and water. It can also be added to everyday products like toothpaste, mouthwash, and drinking water. Fluoride is commonly used to help prevent cavities and tooth decay.

What is community water fluoridation (CWF)?

CWF is a public health practice used in many communities across the U.S. It involves adjusting the amount of fluoride in public drinking water to a level that helps reduce tooth decay. Some water systems naturally have fluoride; others may choose to add it.

Who decides whether to start or stop CWF?

In the U.S., the decision to fluoridate public water systems is made at the state or local level.

In Washington State, the decision to fluoridate drinking water is made at the local level by each public water system or local government.

If a community decides to add fluoride, it must follow the rules set by the Washington State Board of Health (or "Board"). The Washington State Department of Health is responsible for enforcing these rules through its Drinking Water Program.

What is the role of the State Board of Health in CWF?

State law (RCW 43.20.050) requires the Board to set health and safety standards for public water systems to ensure safe and reliable drinking water.

Does the Board of Health require CWF?

No. The Washington State Board of Health does not require or ban water fluoridation. It is up to each public water system or local government to decide whether to fluoridate.

The Board has set an optimal fluoride concentration level of 0.7 milligrams per liter (mg/L) in state rule (WAC 246-290-460). This rule applies to large (Group A) public water systems that choose to fluoridate.

What role does the federal government play in CWF?

The federal government sets national safety standards for fluoride in drinking water under the Safe Drinking Water Act of 1974 (SDWA) through the Environmental Protection Agency (EPA).

Two federal agencies are mainly involved in CWF: the Environmental Protection Agency (EPA) and the United States Public Health Service (USPHS).

The federal government does not require communities to add or remove fluoride from drinking water if levels stay below the EPA's limit of 4.0 milligrams per liter (mg/L). This limit is also called the Maximum Contaminant Level (MCL).

The USPHS recommends a fluoridation level of 0.7 mg/L for water systems that choose to fluoridate.

Communities have made decisions about fluoridation since 1945, when Grand Rapids, Michigan, became the first U.S. city to fluoridate its water.

Other federal agencies, such as the Food and Drug Administration (FDA), regulate fluoride when it is added to consumer products like toothpaste, mouthwash, and dietary supplements.

Which federal agencies are involved in CWF and what do they do?

U.S. Environmental Protection Agency (EPA)

- Regulates fluoride levels in public drinking water under the Safe Drinking Water Act.
- Sets the enforceable safety limit at 4.0 mg/L to protect against health effects from too much fluoride, whether naturally occurring or added in excess of the optimal level.
- Focuses on the level of fluoride that presents a health risk.

U.S. Public Health Service (USPHS)

- Part of the U.S. Department of Health and Human Services (HHS), USPHS includes the Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and Agency for Toxic Substances and Disease Registry (ATSDR).
- Recommends the optimal fluoride level in drinking water (0.7 mg/L) to help prevent tooth decay while minimizing health risks.
- Provides scientific guidance to support state and local public health decisions.

U.S. Food and Drug Administration (FDA)

- Part of HHS that works to ensure the safety and effectiveness of medicines, vaccines, medical devices, and other health-related products. They also oversee the safety of some food, cosmetics, dietary supplements, and more.
- Responsible for making sure fluoride is safe and effective when used in consumer products. This includes toothpaste, mouthwash, fluoride supplements, and other over-the-counter dental products.
- Sets standards for how consumer products are labeled and ensures they meet safety and quality requirements before they are sold to the public.
- Does not regulate fluoride levels in public drinking water.

U.S. Department of Agriculture (USDA)

- Oversees farming, food safety, and nutrition programs.
- Collects and shares data on fluoride levels in food and beverages.
- Supports nutrition and public health guidance that considers fluoride intake from dietary sources.
- Does not regulate fluoride levels in public drinking water or consumer products.

Why is there recent increased attention on CWF and fluoride?

There is growing national attention on fluoridation due to:

- New scientific studies
- A recent federal court ruling
- Ongoing discussions at the federal level

What's happening at the federal level regarding CWF or fluoride?

In April 2025, the EPA announced it would review new scientific data on fluoride health risks, following a court order. This ruling did not ban fluoride or require any immediate changes. As of August 2025, the EPA has not taken formal action or provided updates on this work.

In May 2025, the FDA announced it is beginning the process to possibly remove ingestible fluoride prescription drug products for children from the market. The FDA plans to finish a safety review and collect public feedback by October 31, 2025.

Is anything changing in Washington State regarding CWF?

No. CWF remains a local decision. The Board will continue monitoring federal developments in collaboration with the Department of Health. The Board will also:

- Receive updates on the latest science and evidence from the Department of Health's Fluoride Science Review Panel.
- Provide updates as new information becomes available.

To request this document in an alternate format or a different language, please contact the Washington State Board of Health at 360-236-4110 or by email at wsboh@sboh.wa.gov. TTY users can dial 711.

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Sources of Fluoride in the United States

Fluoride is a mineral that helps keep teeth strong and prevents cavities. Fluoride is found in nature—in water, food, and soil—and is also added to some products to help protect teeth.
Here are the main ways people in the U.S. get fluoride:

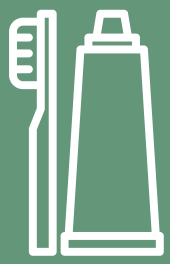


DRINKING WATER

Most fluoride people get in the U.S. comes from drinking water. Some communities have natural fluoride in their water. In others, fluoride is added to help stop tooth decay. The federal government sets a limit for the amount of fluoride in drinking water, and advises on the optimal level. Local and state governments decide whether to add it. Because we use tap water for drinking, cooking, and making drinks, it provides about 60% of the fluoride people get.

TOOTHPASTE AND OTHER DENTAL PRODUCTS

Most toothpaste in the U.S. has fluoride in it. Fluoride is also in some mouth rinses, gels, and varnishes. People, especially kids, can swallow small amounts when they brush or use these products.





BABY FORMULA

Powdered and liquid baby formulas can have fluoride, especially if made with tap water that has fluoride in it. Soy-based formula usually has more fluoride than milk-based formula.

FOODS AND DRINKS

Most foods have only very small amounts of fluoride. Tea (especially black tea) has more fluoride because the tea plant takes it in from the soil. Other foods with some fluoride: coffee, canned shrimp and crab, oatmeal, potatoes, raisins, rice, and some juices.





SUPPLEMENTS AND MEDICATIONS

Some children may be prescribed fluoride drops or tablets by their healthcare provider or dentist if they live in areas without fluoridated water. A few prescription medicines also have small amounts of fluoride, but it's not the main ingredient.

National Institutes of Health. Office of Dietary Supplements - Fluoride Fact Sheet for Health Professionals. April 11, 2025. Accessed August 6, 2025. https://ods.od.nih.gov/factsheets/Fluoride-HealthProfessional/

WASHINGTON STATE BOARD OF HEALTH

Date: August 20, 2025

To: Washington State Board of Health Members

From: Kelly Oshiro, Vice Chair

Subject: Federal Changes in Newborn Screening and Proposed Updates to Washington State's Candidate Condition Review Process– Possible Action

Background and Summary:

The Washington State Board of Health (Board) has the authority under RCW 70.83.050 to adopt rules for screening newborns in Washington for certain inherited conditions. These rules in chapter 246-650 WAC specify the list of conditions included in the Washington State mandatory newborn screening panel.

The Board and Department of Health (Department) use a well-established process to review and consider new conditions for the screening panel. This process is guided by evidence, ethics, equity, and cost-effectiveness. When a condition meets the Board's qualifying assumption, meaning there is enough scientific evidence to assess it using the Board's criteria, the Board partners with the Department to convene a technical advisory committee (TAC) to review the evidence and make a recommendation.

The Board last updated its review process, criteria, and qualifying assumption in late 2024 and early 2025. Updates included:

- Expanding and clarifying the screening criteria,
- Adding a sixth criterion: public health infrastructure readiness, and
- Specifying that any condition recommended by the U.S. Health and Human Services Secretary would automatically meet Washington's qualifying assumption and be reviewed by a TAC within two years.

Since then, major changes have occurred at the federal level. In spring 2025, the U.S. Department of Health and Human Services (HHS) eliminated the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC).[1] [2] For over 20 years, this multidisciplinary committee of experts advised the HHS Secretary on newborn screening policy and recommended conditions for the national Recommended Uniform Screening Panel (RUSP).[3]

Since 2010, the RUSP has served as a national guide for state newborn screening programs, including Washington's. Adding a condition to the RUSP involved a transparent and evidence-based process. Without the ACHDNC, there is currently no federal advisory body to assess or recommend new conditions. This shift places greater

(continued on the next page)

responsibility on states to assess and consider conditions for their programs without federal guidance. It also creates uncertainty for advocates and raises concerns about consistency, coordination, and equity in screening efforts across the country.[1]

While some advocates believe the HHS Secretary may still accept nominations to add new conditions, it is unclear whether there will be changes to the review process. Additionally, two conditions, metachromatic leukodystrophy (MLD) and Duchenne muscular dystrophy (DMD), were under review by ACHDNC before its dissolution. Board and Department staff have met with advocates to discuss possible next steps for these conditions.

Considering these recent changes, I have invited Megan McCrillis, Department staff, and Molly Dinardo, Board staff, to share additional updates about newborn screening at the federal level and present proposed changes to Washington's candidate condition review process and qualifying assumption.

In addition, staff will propose clarifying the Board's authority regarding specimen types, informed by recent experiences with candidate condition reviews and TAC processes.

Recommended Board Action

The Board may wish to consider and amend, if necessary, the following motion:

The Board accepts the proposed updates to the candidate condition review process and qualifying assumption, as presented by Board and Department staff, for the purpose of evaluating conditions for inclusion in Washington's mandatory newborn screening panel.

OR

The Board does not accept the proposed updates to the candidate condition review process and qualifying assumption, as presented by Board and Department staff, for the purpose of evaluating conditions for inclusion in Washington's mandatory newborn screening panel, for the reasons stated by the Board.

Staff

Molly Dinardo, Policy Advisor

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[1] Choudhury S, IV RH. Newborn Screening At Risk: Implications of Disbanding The Advisory Committee on Heritable Disorders In Newborns and Children. May 1, 2025. Accessed August 11, 2025. https://www.healthaffairs.org/content/forefront/newborn-screening-risk-implications-disbanding-advisory-committee-heritable-disorders

[2] Cystic Fibrosis Foundation. Coalition Expresses Concerns About the Elimination of the Advisory Committee on Heritable Disorders in Newborns and Children. May 5, 2025. Accessed August 11, 2025. https://www.cff.org/statements/2025-05/coalition-expresses-concerns-about-elimination-achdnc

[3] Health Resources Administration (HRSA). Advisory Committee on Heritable Disorders in Newborns and Children. Last Reviewed February 2025. Accessed August 11, 2025. https://www.hrsa.gov/advisory-committees/heritable-disorders



Newborn Screening: Federal Changes and Possible Updates to Washington's Process

Molly Dinardo, Board Staff
Megan McCrillis, Department Staff

August 20, 2025

WASHINGTON STATE BOARD OF HEALTH

Recent Updates to Washington State's Process and Criteria

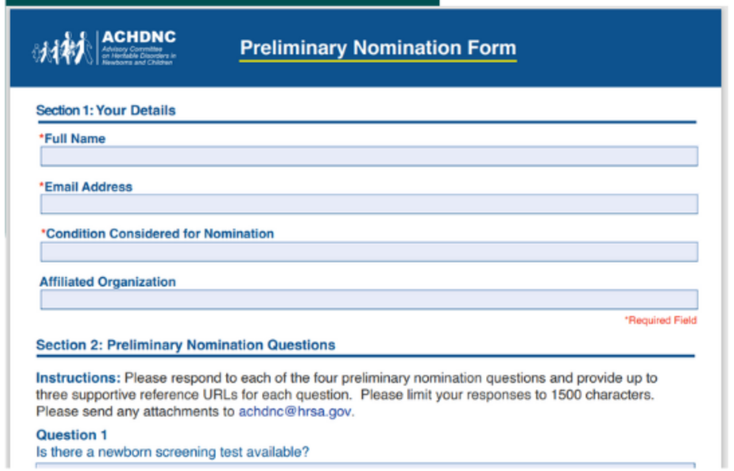
- The Board last updated its review process, criteria, and qualifying assumption in late 2024 and early 2025 for quasi-RUSP alignment.

- Updates included:
  - Expanding and clarifying the screening criteria,
  - Adding a sixth criterion: public health infrastructure readiness, and
  - Specifying that any condition recommended by the HHS Secretary would automatically meet Washington's qualifying assumption and be reviewed by a TAC within two years.



Federal Role in Newborn Screening

Condition nomination



ACHDNC
Advisory Committee on Heritable Disorders in Newborns and Children

Preliminary Nomination Form

Section 1: Your Details

*Full Name

*Email Address

*Condition Considered for Nomination

Affiliated Organization

*Required Field

Section 2: Preliminary Nomination Questions

Instructions: Please respond to each of the four preliminary nomination questions and provide up to three supportive reference URLs for each question. Please limit your responses to 1500 characters. Please send any attachments to achdnc@hrsa.gov.

Question 1

Is there a newborn screening test available?

Advisory Committee on Heritable Disorders in Newborns and Children **(ACHDNC)**

The U.S. Department of Health and Human Services **(HHS)**

The Recommended Uniform Screening Panel **(RUSP)**



WSBOH
Newborn Screening Technical Advisory Committee

Changes in Federal Newborn Screening Condition Recommendation Oversight

- April 2025 – The U.S. Health and Human Services (HHS) Secretary terminates the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC). Evidence-based reviews for Duchenne's Muscular Dystrophy (DMD) and Metachromatic Leukodystrophy (MLD) are disrupted.

- May - July 2025 – American College of Medical Genetics and Genomics (ACMG) forms a RUSP Continuation Task Force and hosts a roundtable to explore a new advisory structure for national newborn screening recommendations.

- **August 2025 –** American Academy of Pediatrics (AAP) publishes evidence-based reviews for DMD and MLD. The Health Resources and Services Administration (HRSA) seeks public comment (through mid-September) on adding DMD and MLD to the RUSP.

- **Ongoing –** HRSA, which previously housed ACHDNC, is not currently inviting nominations for other conditions to the RUSP. They are, however, exploring ways to support the RUSP and the newborn screening system.

Other Federal Developments and Possible Impacts on Newborn Screening

July 2025 – H.R. 1 – One Big Beautiful Bill Act Passed

- Major tax and spending bill that will affect Medicaid and food assistance programs across the country.

Medicaid and Newborn Screening in Washington State

- Currently, Medicaid covers over 40% of births in the state.
- Apple Health (WA's Medicaid program) covers ~2 million people.

Potential Impacts of H.R. 1

- New work requirements and eligibility changes may limit access and reduce Medicaid funding in Washington State.
- 200,000 – 300,000 Washingtonians could lose Medicaid coverage.
- The state could lose billions in federal funding over the next decade.



Learnings from Recent Candidate Condition Requests and TAC Meetings

Congenital cytomegalovirus (cCMV) TAC:

- Screening can be done with three specimen types: dried blood spot, saliva swab, and dried urine filter paper.
- Urine specimens provide the most accurate screening.
- Blood spot screening for cCMV does not meet the Board's newborn screening criteria (sensitivity below 95%).

Board Statutory Authority:

- Under Chapter 70.83 RCW, the Board can only adopt rules for screening infants using *blood specimens*.
- Expanding this authority to include non-blood specimens would require time and resources to explore policy options and feasibility.



Proposed Updates to the Board's Process Document

Clearer call-out of Board authority (blood spot specimens):

- The Washington State Board of Health (Board) has the duty under RCW 70.83.050 to define and adopt rules for screening Washington-born infants for heritable conditions using a dried, blood-spot specimen.

Provide more flexibility given federal uncertainty:

- If a candidate condition is on the Health Resources and Services Administration (HRSA) Recommended Uniform Screening Panel (RUSP), the Board and Department may consider the qualifying assumption met and convene a TAC.



For Board Member Discussion

- Would the Board consider accepting or rejecting these proposed changes to the process and criteria document? Why or why not?

- Do the proposed changes provide enough flexibility for the Board and Department to maintain a state-specific, evidence-based review while considering federal or national recommendations outside of ACHDNC and the RUSP?

- Are there any additional changes you would suggest, or any staff-proposed changes that should be removed?



WASHINGTON STATE BOARD OF HEALTH

THANK YOU

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ACCESSIBILITY AND THE AMERICANS WITH DISABILITIES ACT (ADA)

- The Washington State Board of Health (Board) is committed to providing information and services that are accessible to people with disabilities. We provide reasonable accommodations, and strive to make all our meetings, programs, and activities accessible to all persons, regardless of ability, in accordance with all relevant state and federal laws.
- Our agency, website, and online services follow the Americans with Disabilities (ADA) standards, Section 508 of the Rehabilitation Act of 1973, Washington State Policy 188, and Web Content Accessibility Guidelines (WCAG) 2.0, level AA. We regularly monitor for compliance and invite our users to submit a request if they need additional assistance or would like to notify us of issues to improve accessibility.
- We are committed to providing access to all individuals visiting our agency website, including persons with disabilities. If you cannot access content on our website because of a disability, have questions about content accessibility or would like to report problems accessing information on our website, please call (360) 236-4110 or email wsboh@sboh.wa.gov and describe the following details in your message:
    - The nature of the accessibility needs
    - The URL (web address) of the content you would like to access
    - Your contact information

We will make every effort to provide you the information requested and correct any compliance issues on our website.



WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Panel PROCESS & CRITERIA

PROCESS TO EVALUATE CONDITIONS FOR INCLUSION IN THE REQUIRED NEWBORN SCREENING PANEL

Last updated ~~March 14~~August 20, 2025

The Washington State Board of Health (Board) has the duty under RCW 70.83.050 to define and adopt rules for screening Washington-born infants for heritable conditions using a dried-blood spot specimen. Chapter 246-650-020 WAC lists conditions for which all newborns must be screened. Members of the public, staff at the Department of Health (Department), and/or Board Members can request that the Board review a particular condition for possible inclusion in the newborn screening (NBS) panel.

To determine which conditions to include in the NBS panel, the Board convenes a newborn screening technical advisory committee (TAC) to evaluate candidate conditions using guiding principles and an established set of criteria.

This document describes the Qualifying Assumption, Guiding Principles, and Criteria the Board has approved to evaluate conditions for possible inclusion in the newborn screening panel. The Board and Department apply the qualifying assumption. The Board-appointed Newborn Screening TAC applies the following three guiding principles and evaluates the criteria to make recommendations to the Board on which condition~~(~~s~~)~~ to include in the state's required NBS panel.

QUALIFYING ASSUMPTION

Before the Board convenes a TAC to review a candidate condition against the newborn screening criteria, staff should complete a preliminary review to determine whether sufficient scientific evidence is available to apply the criteria for inclusion. This is called~~,~~ ~~which is~~ the qualifying assumption.

If the candidate condition is on the Health Resources and Services Administration (HRSA) Recommended Uniform Screening Panel (RUSP), the Board and Department ~~will~~ may consider the qualifying assumption met and convene a TAC.

A note on the RUSP: The RUSP is a list of conditions that the Secretary of the Department of Health and Human Services (HHS) recommends states screen for as part of their newborn screening programs. Once the HHS Secretary recommends a new condition, the Board and Department may~~will~~ review it for possible inclusion in the Washington NBS panel within two years of the recommendation.

Conditions pending RUSP Review or Previously Denied for the RUSP: RCW 34.05.330 of the Administrative Procedures Act (APA) allows any person to petition a state agency to adopt, repeal, or amend any rule within its authority. Agencies must respond to the petitioner within 60 days. If the agency accepts the petition, it must initiate rulemaking. An agency can deny the request for rulemaking, and in doing so, it must explain its reasons and, if appropriate, describe alternative steps it is prepared to take.

If the Board receives a request to review a condition for possible inclusion on the panel—including a petition for rulemaking under RCW 34.05.330—and the ~~regarding a candidate~~ condition is currently under review for the RUSP, the Board will wait until the ~~federal~~ committee ~~finishes~~ completes its review and the HHS Secretary makes a final decision before convening a TAC.

For petitions involving conditions that have already been reviewed and denied inclusion on the RUSP, the Board will instruct staff to work with the petitioner to determine if concerns raised during the federal review have been addressed before recommending the Board convene a TAC to review the condition.

THREE GUIDING PRINCIPLES

Three guiding principles govern all aspects of the evaluation of a candidate condition for possible inclusion in the NBS panel.

- ~~Decision~~ A decision to add a screening test should be driven by evidence. For example, test reliability and available treatment have been scientifically evaluated, and those treatments can improve health outcomes for affected children.
- All children who screen positive should have reasonable access to diagnostic and treatment services.
- Benefits of screening for the disease or ~~/~~condition should outweigh harm to families, children, and society.

CRITERIA

1. **Available Screening Technology:** Sensitive, specific, and timely tests are available that can be adapted to mass screening.
    - The sensitivity of the screening test is estimated to be ≥95%.
    - The specificity of the screening test is considered acceptable based on the estimated number of false positive results and their potential impact on the families, healthcare system, and newborn screening program.
    - A timely test is one that enables intervention before irreversible harm develops, within the current standard timeframes for specimen collection, receipt, testing, and reporting.
    - There is adequate peer reviewed evidence to evaluate this criterion.

2. **Diagnostic Testing and Treatment Available:** Accurate diagnostic tests, medical expertise, and effective treatment are available for evaluation and care of all infants identified with the condition.
    - A diagnostic test accurately identifies who needs treatment and is readily available to all newborns screened.
    - The available treatment is effective in reducing morbidity or mortality and outweighs any risks or harms of the treatment.
    - The medical expertise needed to diagnose and care for those with a positive newborn screen is reasonably available to all newborns screened.
    - The appropriate consultants and treatment centers have been identified and have capacity for the expected increase in diagnostic testing and/or referrals.
3. **Prevention Potential and Medical Rationale:** The newborn identification of the condition allows early diagnosis and intervention.
    - There is sufficient time between birth and onset of irreversible harm to allow for diagnosis and intervention.
    - The condition must have an onset form that occurs in infancy (within the first year of life); newborn screening is not appropriate for conditions that only present after the first year of life.
    - The benefits of detecting and treating infantile-onset forms of the condition (within one year of life) balance the impact of detecting later onset forms of the condition.
    - There is adequate evidence of acceptable quality to evaluate this criterion.
4. **Public Health Rationale:** Nature of the condition justifies population-based screening rather than risk-based screening or other approaches.
    - All available risk-based screening tools for the condition have been considered and are found to be inferior to universal newborn screening.
    - There is adequate evidence of acceptable quality to evaluate this criterion.

5. **Cost-benefit/Cost-effectiveness:** The outcomes outweigh the costs of screening. All outcomes, both positive and negative, need to be considered in the analysis.
    - The economic analysis considers:
        - The prevalence of the condition among newborns.
        - The positive and negative predictive values of the screening and diagnostic tests.
        - Variability of clinical presentation by those who have the condition.
        - Dollar values for costs and benefits of screening vs. no screening.
    - The impact of ambiguous results, adverse effects, or unintended consequences of screening, such as psycho-social or economic impacts on the family and medical system, must also be considered.
    - The results of the economic analysis shows that the outcomes, financial or otherwise, outweigh the costs of screening.
    - There is adequate evidence of acceptable quality to evaluate this criterion.
6. **Public Health Infrastructure Readiness:** The Newborn Screening Program's capacity to implement screening within a reasonable timeframe has been considered.
    - The systems and staffing necessary to perform the test and report screening results have been identified.
    - Resources needed to implement short/long term follow up protocols by the newborn screening program have been identified.
    - Accessibility to treatment for anyone diagnosed with the condition is considered acceptable based on the frequency of treatment needed.

Washington State Health Care Authority

Apple Health (Medicaid) in Washington

On July 4, 2025, Congress signed into law a continuing resolution for the federal budget. It contains numerous changes that impact Apple Health and the individual market. (Apple Health is what we call Medicaid in Washington State.) The Health Care Authority (HCA) and our partners are still assessing the full scope of impacts to Apple Health but anticipate significant administrative changes and new state costs associated with implementation.

Impacts of the federal budget on Apple Health

- **Apple Health provides health coverage to almost 2 million Washingtonians.**
- Between 200,000 and 320,000 Washingtonians are projected to lose Medicaid coverage because of the federal budget.
- Washington is projected to lose billions in federal Medicaid funding between 2025–2034.

Major Medicaid policies and timelines (based on enacted budget)

	Policy	Effective Dates
Restricts payment for protected health services	Restricts federally funded Medicaid payments for 1 year to nonprofit organizations that primarily engage in family planning services or reproductive services and provide abortion services. Likely to impact over $11 million in funding.	Effective for 1 year, **from date of enactment** (July 4, 2025)
Funding for non-citizens	Changes Medicaid eligibility for refugee, asylee, and other non-citizen adults.	**Oct. 1, 2026**
Work requirements	Establishes work requirements as a new condition of Medicaid eligibility for adults aged 19-65 who receive full coverage. This makes coverage based on working, training, or doing community engagement 80 hours per month. Includes certain categorical exemptions.	**Dec. 31, 2026**, with option to apply for waiver to implement Dec. 31, 2028
Rural health funding	Allocates $10 billion annually to states, which can be used to support rural health transformation projects with a focus on promoting care, supporting providers, investing in technology, and assisting rural communities.	States can apply in **2025**; funding from 2026–2030
Increases the frequency of eligibility redeterminations	Requires states to redetermine eligibility for adults enrolled through Medicaid expansion every 6 months, instead of every 12 months.	**Dec. 31, 2026**
Retroactive coverage	Shortens period of retroactive coverage eligibility from 3 months to 1 month for adults and 2 months for other Medicaid and CHIP applicants.	**Jan. 1, 2027**
Restricts new state-directed payments (SDPs) from exceeding Medicare payment levels	Requires existing SDPs for hospital and nursing facility services and services provided at an academic medical center to reduce by 10% per year, beginning in 2028 until they reach Medicare levels.	10% reductions begin in **2028**
Cost-Sharing	Requires adults to pay cost-sharing of up to $35 for many services. Excludes primary care, behavioral health, emergency services, and services rendered in certain rural settings from the requirement.	**Oct. 1, 2028**
Address verification	Changes requirements for address verification.	**Oct. 1, 2029**
Removes good-faith waivers related to erroneous payments	Removes ability to waive federal penalties for a state's good-faith efforts to correct erroneous excess Medicaid payments under the Payment Error Rate Measurement (PERM) program and other state and federal audits.	**Oct. 1, 2029**

WASHINGTON STATE BOARD OF HEALTH

Date: August 20, 2025

To: Washington State Board of Health Members

From: Kelly Oshiro, Board Vice Chair

Subject: Newborn Screening, Wilson Disease TAC Recommendations

Background and Summary:

The Washington State Board of Health (Board) has the authority under RCW 70.83.050 to adopt rules for screening newborns in Washington for certain inherited conditions. These rules in chapter 246-650 WAC specify the list of conditions included in the Washington State mandatory newborn screening panel.

On July 31, 2024, the Board received a rulemaking petition to add Wilson disease to the state's mandatory newborn screening panel. The petition stated that early detection of Wilson disease allows for timely treatment and can prevent serious damage to the liver and nervous system.[1]

Wilson disease is a rare inherited disorder caused by mutations in the ATP7B gene, which normally encodes the protein that helps remove excess copper from the body. When the protein doesn't function properly, copper builds up, first in the liver, then in the blood, brain, and other organs. This can lead to serious, sometimes life-threatening complications.[2] [3]

Symptoms of Wilson disease vary widely. Some people develop liver-related symptoms, such as jaundice, swelling, or fatigue. Others may first experience neurological or behavioral symptoms, including speech or coordination challenges.[1] [2] [3] Symptoms can appear at any time between ages 3 and 70.

Currently, no other state screens newborns for Wilson disease, and it is not included on the federal Recommended Uniform Screening Panel (RUSP). However, Washington State's newborn screening laboratory has partnered with Key Proteo on a pilot project that tested 50,000 de-identified residual samples to assess the feasibility of population-based screening.[4]

On June 17, 2025, the Board and Department of Health convened a technical advisory committee (TAC) to evaluate Wilson disease against the Board's newborn screening criteria. The committee reviewed information on the condition's natural history, diagnostic and treatment options, available screening technology, cost-effectiveness, and public health system readiness. The TAC voted on each criterion and provided an overall recommendation to the Board.

(continued on the next page)

I have invited Megan McCrillis, Policy Analyst for the Department of Health's Newborn Screening Program, and Molly Dinardo, Policy Advisor to the Board, to present information from the Wilson Disease TAC for Board Member consideration.

Recommended Board Actions:

The Board may wish to consider one of the following motions:

The Board directs staff to file a CR-101 to initiate rulemaking for chapter 246-650 WAC to consider adding Wilson Disease to the Washington State newborn screening panel.

OR

The Board determines that Wilson Disease should not be considered for addition to the newborn screening panel at this time for the reasons stated by the Board.

Staff

Molly Dinardo, Policy Advisor

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[1] National Institutes of Health National Library of Medicine. Wilson Disease: GeneReviews. Published January 2023. Accessed July 22, 2024. https://www.ncbi.nlm.nih.gov/books/NBK1512/#wilson

[2] National Organization for Rare Disorders. Wilson Dease. Published March 2018. Accessed July 22, 2024. https://rarediseases.org/rare-diseases/wilson-disease

[3] National Institutes of Health National Library of Medicine. Wilson Disease: StatPearls . Published August 2023. Accessed July 22, 2024. https://www.ncbi.nlm.nih.gov/books/NBK441990/

[4] Key Proteo. Key Proteo Pilots Newborn Screening in Washington State. Published Mar 2022. Accessed July 22, 2024.

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Newborn Screening
Technical Advisory Committee (TAC) Recommendations for Wilson Disease

Molly Dinardo, Board Staff
Megan McCrillis, Department Staff

August 20, 2025

WASHINGTON STATE BOARD OF HEALTH

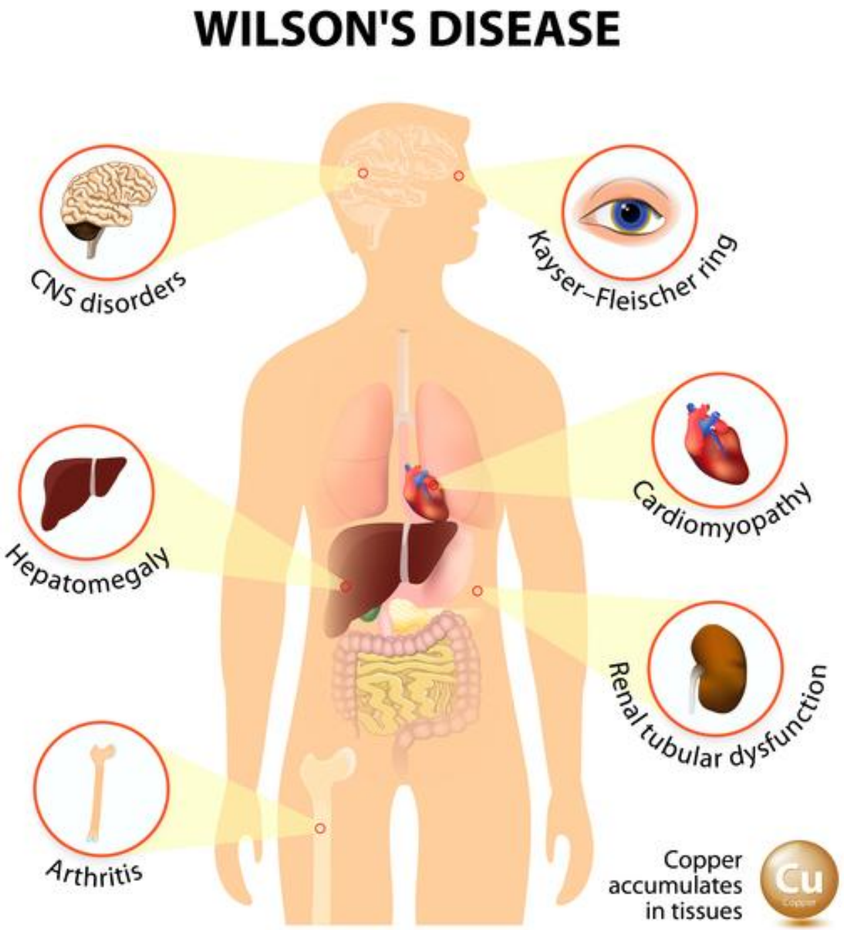
Background

- In August 2024, Key Proteo submitted a petition to the Board to consider adding Wilson Disease to Washington’s newborn screening panel.
- Wilson Disease is a rare, inherited metabolic disorder that prevents the body from eliminating excess copper.
- The condition is not currently screened for in any U.S. state and has not been reviewed for inclusion on the Recommended Uniform Screening Panel (RUSP).
- Seattle Children’s Hospital is home to a Wilson Disease Center of Excellence.
- From 2022 to 2024, the Washington State Newborn Screening Laboratory partnered with Key Proteo on a prospective pilot study to evaluate screening for Wilson Disease.
- The Board convened a Technical Advisory Committee (TAC) to review the condition on June 17, 2025.



Wilson Disease Overview

- Wilson Disease (WD) is a rare inherited disorder of copper metabolism.
- The condition is caused by changes in the ATP7B gene that reduce or eliminate the protein responsible for removing excess copper.
- About 1 out of 30,000 people are affected by WD in the U.S.
- Wilson Disease symptoms typically develop between the ages of 5 and 35, but cases have been reported in children as young as 3 and adults up to age 70.
- Common symptoms include:
  - Liver dysfunction
  - Neurologic impairment (e.g., tremors, movement issues)
  - Psychiatric conditions (e.g., depression, personality changes)



Source: Medlineplus.gov/genetics/condition/Wilson-disease

Available Screening Technology

- Screening method detects low levels of ATP7B-related peptides in dried blood spots:
  - Uses LC-MS/MS technology (already in use in WA's NBS lab)
  - Requires a new peptide-based application of existing equipment

- Validation study results:
  - 32 confirmed cases: 100% screen positive
  - Sensitivity ~95.5%
  - Sequencing of borderline samples showed very low false-negative risk

- Pilot study in WA (30,024 newborns):
  - 1 possible true positive
  - 3 false positives (~8/year)
  - DNA sequencing needed for confirmation

- Turnaround time:
  - Results available within 1–2 days
  - Screening is timely enough to allow intervention before treatment typically begins (~18 months of age)



Diagnostic Testing and Available Treatment

- Diagnostic testing:
  - Blood tests: Low ceruloplasmin levels
  - Abnormal liver function tests
  - Urine tests: Elevated 24-hour urinary excretion
  - Imaging/biopsy: Liver biopsy and/or brain imaging (if needed)
  - Genetic testing: ATP7B mutation analysis to confirm diagnosis

- Treatment options:
  - Chelation therapy to remove excess copper
  - Zinc supplementation to block copper absorption
  - Low-copper diet
  - Liver transplant in severe or advanced cases



Cost Benefit and Cost Effectiveness

	No Screening	Universal Screening	Shift
Deaths	0.075	0.003	-0.072
Liver Transplants	0.114	0.005	-0.109
Neurologic Cases	0.54	0.02	-0.52
Cases with Early Treatment	0.16	1.92	+1.76

Cost Benefit and Cost Effectiveness

Benefits	Value
Benefit of Lives Saved	$943,106.44
Benefit of Liver Transplants Averted	$18,711.88
Benefit of Neurologic Cases Averted	$1,193,011.19
Benefit of Less Treatment Costs	-$51,493.20
Total Benefits	$2,103,336.31

Costs	Value
Cost of Screening	$1,511,479.04 ($18.89 per baby)
Cost of False Positives	$15,986.81
Cost of Treatment	$290,786.32
Total Costs	$1,818,252.17

Benefit/Cost ratio	1.16
Net Benefit	$285,084.14

Public Health Infrastructure Readiness

Systems:
- Laboratory equipment: (2) Xevo LC-MS/MS

Ongoing Staffing:
- 1 Full Time Equivalent, Chemist 2 (frontline lab staff, to perform the test)
- 0.1 Full Time Equivalent, Chemist 3 (lab supervisor, to provide testing and reporting oversight)

Startup Staffing:
- ~2 months of Chemist 3 work to develop workflow and validate new equipment and methodology

Newborn Screening Follow-up Staffing
- 0.1 Full Time Equivalent, Health Services Consultant 2 (ongoing)
- ~40 hours of Epidemiologist 2 work (start-up)

All the systems and staffing needs noted above were included in the cost-benefit analysis.

TAC Voting and Recommendations

Criteria Evaluation	Yes	No	Unsure
Available Screening Technology	15	0	1
Diagnostic Testing and Treatment	16	0	0
Prevention Potential and Medical Rationale	14	0	2
Public Health Rationale	15	0	1
Cost-benefit / Cost-effectiveness	14	0	2
Public Health Infrastructure Readiness	14	1	1

Overall Recommendation	Vote
I recommend the Board add Wilson Disease to the list of conditions for which all Washington infants must be screened…	13
I do not recommend the Board add Wilson Disease to the list of conditions for which all Washington infants must be screened…	0
I do not recommend the Board add Wilson Disease at this time; I recommend the Board revisit Wilson Screening at a future date…	1
No response or missing response	2

Board Member Discussion and Next Steps

- For discussion: Does the Board agree with the TAC's recommendation for Wilson Disease? Why or why not?

- **Possible Action:** The Board may consider the following…
  - Direct staff to initiate rulemaking to include Wilson Disease in the newborn screening panel.

    OR
  - Determine that Wilson Disease should not be considered at this time for reasons stated by the Board.



WASHINGTON STATE BOARD OF HEALTH

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Washington State Department of HEALTH

WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Technical Advisory Committee (TAC)

Wilson Disease (WD) Overview

ABOUT THE CONDITION

- WD is a rare inherited disorder of copper metabolism.
- WD is caused by changes in the *ATP7B* gene that reduce or eliminate the protein responsible for removing excess copper.
- Copper then accumulates in the body, especially in the liver and brain.
- WD may be detected via tandem mass spectrometry. The technology application will be reviewed at the TAC meeting.
- About 1 out of 30,000 individuals are affected by WD.

SYMPTOMS

- Most individuals with WD first develop symptoms between the ages of 5 and 35.
- WD symptoms vary between individuals, but can include:
    - Hepatic symptoms such as inflammation, cirrhosis, or liver failure
    - Neurological dysfunction such as movement disorders, tight muscles, or difficulty swallowing
    - Psychiatric disorders such as depression or anxiety.
- In about half of those with WD, the liver is the only major organ system affected. Other patients with WD may have neurological or psychiatric symptoms only or a combination of these systems.
- Many individuals with WD exhibit Kayser-Fleischer (KF) rings, which are copper deposits in the cornea of the eye.

DIAGNOSIS

- WD is often diagnosed years after symptoms begin due to their non-specific nature. Diagnosis usually involves a physical exam and tests like ceruloplasmin levels, 24-hour urinary copper excretion, liver function tests, or a liver biopsy.
- Genetic testing for changes in the *ATP7B* gene is the gold standard for confirming a diagnosis.

TREATMENT

- Treatment for WD can include:
    - Preventing excess copper accumulation by limiting dietary intake of copper
    - Removing excess copper from the body through chelation therapy drugs
    - Blocking the absorption of copper in the intestinal tract through zinc acetate medication

- Lifelong treatment is necessary to manage WD. Nonadherence may result in severe health complications, including liver failure or death.
- Liver transplantation may be necessary for those with severe liver damage or failure.

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Wilson Disease Association. (2021, August 30). *Living with Wilson disease*. https://wilsondisease.org/living-with-wilson-disease/

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Considering adding Wilson disease to the Washington State Newborn Screening Panel

A narrative of an economic analysis for the Department of Health, State Board of Health, and technical advisory committee

2025

Proposed Rule and Brief History

The State Board of Health (Board) is authorized by RCW 70.83.050 to adopt rules and regulations relating to congenital newborn screening (NBS). The Board established rules under chapter 246-650 WAC regarding which conditions to include on the NBS panel. RCW 70.83.020 grants the Board authority to identify which screening the Department of Health (Department) is required to perform for all infants in the state. RCW 70.83.030 tasks the Board with adopting rules related to the reporting of heritable and metabolic disorders to the Department.

In 2024, Dr. Sihoun Hahn of Key Proteo, Inc. petitioned the Board to institute newborn screening for Wilson disease. After reviewing the available evidence, the Board directed Department staff to convene a multidisciplinary technical advisory committee (TAC) to consider adding Wilson disease to the list of mandated NBS conditions in WAC 246-650-010 and WAC 246-650-020. The TAC evaluated Wilson disease against six Board-approved criteria for potential inclusion in the NBS panel in June 2025. After reviewing the evidence, most TAC members agreed Wilson disease met all six screening criteria and voted to recommend its addition to the panel (one member voted no). The Board will review the comments and votes of the TAC at the August 2025 Board meeting and vote on whether to add the condition to the newborn screening panel.

Overview and Background – Wilson disease

Wilson disease (WD) is a rare autosomal recessive disorder of copper metabolism that is caused by changes in the *ATP7B* gene, which typically produces the protein responsible for elimination of excess copper from the body. Without this protein, copper accumulates in the body, particularly in the liver and brain.

WD symptoms often present between the ages of 5 and 35. Symptoms vary between individuals but can include hepatic symptoms such as inflammation, cirrhosis, or liver failure, neurological dysfunction such as movement disorders, tight muscles, or difficulty swallowing, and psychiatric disorders such as depression or anxiety. In about half of those with WD, the liver is the only major organ system affected. Other patients with WD may have neurological or psychiatric symptoms only, or a combination of these systems affected.

Early identification of WD can allow for treatment, which may include preventing excess copper accumulation by limiting dietary intake of copper, removing excess copper from the body through chelation therapy drugs, or blocking the absorption of copper in the intestinal tract through zinc acetate medication. Lifelong treatment is necessary to manage WD. Benefits of

early treatment include prevention of premature mortality, symptoms, and organ damage that can lead to liver transplant or severe neurologic symptoms. Nonadherence to a prescribed treatment plan may result in severe health complications, including liver failure or death. Liver transplantation may be necessary for those with severe liver damage or failure.

WD is present in approximately one in 40,000 births. At present, no other U.S. state or country universally screens newborns for WD at birth; nor has WD been petitioned to the federal Recommended Universal Screening Panel (RUSP).

Diagnostic testing for WD is primarily based on sequencing of the *ATP7B* gene to look for disease-causing changes.

Overview of Benefit-Cost Analysis

The model structure was developed in 2025 by the Washington NBS program and presented to the WD NBS TAC on June 17, 2025. It will be presented to the Board on August 20, 2025.

The following summary explains the benefit-cost analysis performed in consideration of adding WD to the mandatory NBS panel. The economic model estimates the benefits and costs of universal NBS for WD and the analysis compares these benefits and costs to what is currently happening in the absence of screening. The calculations for this analysis were done in a spreadsheet (available upon request) and includes a "No Screening Model" (upper section) with a "Universal Screening Model" (lower section) (Figure 1).

The analysis is from the societal perspective, in which all costs for providing services are estimated, regardless of who pays the costs. Point estimates and ranges for input variables were derived from primary literature, data from an NBS pilot study, and consultations with experts. The analysis estimates a benefit-cost ratio of 1.16. This means that for every dollar of costs for universal NBS for WD, there will be approximately $1.16 worth of benefit. The annual net benefit for universal WD screening in Washington State was estimated to be $293,448.372.

Process of Cost-Benefit Analysis

The first step is to estimate the number of babies born with WD. We used information from a 2020 publication that estimated the prevalence of WD to be between 1:30,000 and 1:50,000 (1). We chose to use the midpoint estimate of 1:40,000 births and used one year of babies born for this analysis. These estimates predict about two babies born with WD each year in Washington State.

The next step is to find out which newborns will be diagnosed early and benefit from intervention in both sections of the model. In the No Screening Model, a small percentage of newborns will be diagnosed early, even in the absence of screening because they have a positive family history of WD. In contrast, the Universal Screening Model uses the sensitivity of the screening test to diagnose babies early. The sensitivity is the ability of the test to correctly identify newborns with WD. The model predicts that each year there will be about 1.91 babies with WD identified early through universal screening, compared to 0.16 babies identified early based on family history in the no screening model.

Next, we compare the medical outcomes for those diagnosed early versus late with WD. The morbidity estimates are the percentages of babies that would require a liver transplant or develop neurologic symptoms later in life due to disease progression from late-identified WD. The mortality estimates are the percentages of babies we expect would have a decreased life expectancy based on delayed diagnosis and treatment. This includes mortality related to disease progression as well as mortality associated with complications of liver transplant.

The previous steps result in a decision-tree model. The next step is to walk through each branch of the decision tree to produce estimates of the number of newborns that will fall into each type of outcome. To do this, we multiply the estimated percentage or rate at each branch by the number of newborns affected.

Next we compare the medical outcomes of each model. We subtract the numbers of deaths and liver transplants in the Universal Screening Model from the No Screening Model to find the shift in numbers; the result is the difference made by screening. We also calculate the additional infants that will receive diagnostic testing and the number of cases with neurologic symptoms avoided by screening.

We then assign monetary benefits to different outcomes. The value placed on saving a life comes from the U.S. Department of Health and Human Services (2). The value of avoiding a liver transplant includes the cost of the medical procedure, pre- and post- op care, and cost of anti-rejection medications for life. It also includes the value of lifetime disability payments for the proportion of those who are unable to work post-transplant. The value of avoiding the development of neurologic symptoms is based on the expert opinion of annual severe disability support costs.

After the benefits, costs are estimated. The estimated cost for universal NBS is $18.89 per baby, which covers the cost of the laboratory test, staffing, and other ongoing costs. There is also a

one-time start-up cost estimate of $31,411.62 to validate the laboratory test and create follow-up procedures. Costs also include the additional diagnostic testing introduced through universal screening for false positive cases, or those babies who do not have WD but screen positive. They will require DNA sequencing to rule out WD. The final cost estimate is the cost for treatment, which is based on lifetime zinc acetate treatment and lab monitoring.

The final step is to add up all the benefits and the costs. The benefits are divided by the costs to produce a benefit-to-cost ratio. The base ratio of NBS for WD is 1.16. This means that for every dollar of costs to provide universal WD screening, there will be an estimated $1.16 worth of benefits. Another metric to consider is the net benefit. The annual net benefit for universal WD screening estimate is $293,448.37.

Technical Explanation of Model Parameters

We chose numbers for a base case analysis. If we had several estimates from the published data, we used an average, the middle value, the most recent estimate, or the one that most closely reflected the population of Washington State. Note: the spreadsheet we used calculates the percentages and estimates, which have in some instances been rounded for simplicity. Subsequent calculations are unaffected by this rounding, so sometimes the numbers appear to not match perfectly.

- ***Birthrate.*** This analysis is for a hypothetical birth cohort of **80,000** babies (cells B10 and B41), which is the number of babies expected to be screened per year in Washington State. This number is based on the births reported to the WA NBS program in 2023.
- ***Prevalence.*** The prevalence used was **one WD case per 40,000 births** (cells D10 and D32), which is the prevalence reported by Damgaard Sandahl et al (1). This predicts two babies (cells E10 and E32) born with WD in Washington each year.
- ***Percent of babies with WD with a positive family history.*** These babies will be treated early because of a family history of the condition. The estimate for this parameter (**8%,** cells G5 and H37) was an average of findings in the primary literature and the estimate from the population seen by the local WD Center for Excellence (3-7).
- ***Sensitivity****.* The sensitivity, or the ability of the screen to correctly identify babies with WD, is estimated at **95.6%** (cell F29) for universal dried bloodspot screening. This value is the average of the sensitivity estimates derived from a feasibility and pilot study (8-9). The sensitivity value predicts **1.91** true positives (cell G29) identified early and **0.089** false negatives (cell G35) or missed cases of WD per year. True positive babies will need DNA testing to confirm the diagnosis of WD.

- ***Specificity.*** The specificity, or the ability of the screen to correctly identify babies who do not have WD, is estimated at **99.99%** (cell F53) for universal screening. This value is an estimate from the pilot study (8). The specificity value predicts eight false positives per year (cell G46). False positive babies will also need DNA sequencing to rule out WD.
- ***Difference in mortality attributed to progressive WD.*** The mortality estimate for early identified cases of WD (**0%**, cells J4, K29, and K36) is from the literature and expert opinion (10). For those cases of WD that are not diagnosed and treated until the development of symptoms there is an estimated mortality rate of **3.5%** (cells N17 and N47). This estimate comes from the literature (7).
- ***Percent of babies who will require liver transplantation.*** Progressive organ damage caused by late detected WD can require liver transplantation. The estimate for late diagnosed babies that will need a liver transplant is **6.2%**, (cells J14, and K42). This estimate is derived from primary literature (11). For those who are diagnosed and treated presymptomatically, the base assumption is that **0%** will require liver transplants (10).
- ***Liver transplant-related mortality.*** Liver transplantation is a procedure that carries some risk. The mortality estimate for the year following liver transplant is **13%**, (cells N10 and N41). This estimate is derived from primary literature (12-14).
- ***Percent of babies who will develop neurologic symptoms.*** Progressive organ damage caused by late detected WD can lead to neurologic symptoms that, compared to hepatic symptoms, are less amenable to treatment and have a greater negative impact on quality of life. The estimate for late diagnosed babies that will develop neurologic symptoms is **32.2%**, (cells Q19, and Q49). This estimate is derived from primary literature (15). For those who are diagnosed and treated presymptomatically, the base assumption is that **0%** will develop neurologic symptoms (6).

The next step is to evaluate the differences between the models to quantify the benefits and costs of screening. This is done by determining the sum of the following outcomes in the model and calculating the differences made between No Screening and Universal Screening Model.

- ***Shift in mortality.*** The number of deaths estimated are **0.075** (cell W2) in the no screening model and **0.003** (cell W25) in the universal screening model. The anticipated number of deaths averted in each birth cohort through universal NBS is **0.072** (cell W41).
- ***Shift in number of liver transplants.*** The number of liver transplants estimated are **0.114** (cell W4) in the no screening model and **0.005** (cell W27) in the universal screening model. The anticipated number of liver transplants averted in each birth cohort through universal NBS is **0.109** (cell W44).

Benefits are estimated next.

- ***Value of lives saved.*** Per the U.S. Department of Health and Human Services, the value of a life estimate is **$13,234,472.25** (cell W42) (2). The total value of lives saved is the number of deaths averted, multiplied by the value of a life (**$946,950.97,** cell W43) for universal NBS.
- ***Value of liver transplants averted.*** Because an estimated 63% of individuals who undergo liver transplantation are left unable to work, there is value in avoiding liver transplants, beyond the cost of the actual procedure (16). The average annual social security disability payment estimate is $21,093.36 (17). A lifetime of disability payments, applied to 63% of averted liver transplants and discounted, gives a total value of **$18,788.16** (cell W45) in the newborn screening model.
- ***Value of severe disability attributed to neurologic symptoms saved.*** Based on the expert opinion of a health economist, the annual cost of severe disability support is **$150,000.00** (cell W19). **$1,197,874.44** (cell W46) is the total adjusted value of averted severe neurologic disability due to early detection and treatment.
- ***Total benefits of Newborn Screening Model.*** The total benefits of universal screening for each birth cohort (**$2,111,910.45**, cell W48) is the total value of early identification and treatment.

Then, costs are estimated.

- ***Cost of screening.*** The estimated cost of WD NBS testing is **$18.89** (cell B46) per baby for universal screening. Costs for universal newborn screening include staffing for laboratory and follow-up services, new instrumentation and kits, and clinical support. The total cost for WD newborn screening is the birthrate multiplied by cost per baby is **$1,511,479.04** (cell W34).
- ***Costs of diagnostic testing for false positives.*** Babies with false positive screens (those who screen positive but do not have WD) will need diagnostic testing to rule out WD. The estimated cost for diagnostic testing is **$2,000.00** per baby (cell W36); this is the cost for *ATP7B* gene sequencing through Seattle Children's Hospital Laboratory. The total costs of diagnostic testing annually are estimated to be **$15,986.81** (cell W37).
- ***Costs of treatment.*** Babies who are diagnosed with WD shortly after birth will receive twice annual lab monitoring that includes a 24-hour copper (and zinc if applicable), Complete Blood Count, Liver Panel, Serum Copper, and Serum Ceruloplasmin. They will also begin treatment with zinc acetate at approximately 18 months of age. Both the lab monitoring and zinc treatment will be lifelong. The adjusted lifetime cost of treatment

for early identified babies estimate is **$146,697.89** (cell W14). For those with WD who are not diagnosed or begin treatment after the development of symptoms, but who do not require liver transplantation or experience premature mortality, the adjusted lifetime treatment cost estimate is **$90,962.76** (cell W13), which is also based on lifetime twice-annual lab monitoring and zinc acetate treatment. For those identified late who experience early mortality due to disease progression, the adjusted lifetime treatment cost estimate is **$39,731.71** (cell W15). For those who are diagnosed late and undergo a liver transplant with early mortality due to that procedure, the adjusted lifetime cost of treatment is **$513,061.36** (cell W16). This includes the cost of zinc acetate and the cost of the liver transplant with pre- and post-op care and medications. Finally, the adjusted, lifetime treatment cost for those who are diagnosed late, undergo liver transplantation, and survive is **$546,808.16** (cell W17). This includes the cost of zinc acetate leading up to transplant, the cost of the transplant procedure, and the cost of anti-rejection medication for life following transplant. The total costs of treatment in the No Screening model is **$239,293.12** (cell W9) and **$290,996.23** (cell W32) in the Universal Screening model. The difference in treatment costs if universal screening were to be implemented is **-$51,703.11** (cell W47), meaning that treatment costs are estimated to increase with universal screening, due to the identification of more cases, with lifelong treatment.

- ***Total costs of Newborn Screening Model.*** The total annual cost of WD screening is the sum of the ongoing costs of screening, diagnostic testing, and treatment, plus the cost of one-time, start-up activities to prepare the NBS laboratory and follow-up team. The annual ongoing cost for universal screening estimate is **$1,818.462.08** (cell W38) and the one-time startup costs for universal screening estimate is **$31,411.62** (cell W35).
- ***Net Benefit.*** The net benefit is the amount of money saved each year by adding screening and is the total costs subtracted from the total benefits. For universal screening, **$2,111,910.45** minus **$1,818.462.08** gives a net benefit of **$293,448.37** (cell W53).

Finally, the ratio of benefits to cost is calculated. Any ratio greater than 1 signifies that the benefits outweigh the costs.

- ***Benefit-cost Ratio.*** **$2,111,910.45** of benefits divided by **$1,818.462.08** of costs yields a benefit-cost ratio of 1.16 (cell W51).

After completing the base case benefit-cost ratio, we performed a one-way sensitivity analysis to evaluate how the benefit-cost ratio changes when estimates for the parameters are individually varied and all others remain constant.

- ***Sensitivity analysis.*** Table 1 contains three estimates for each parameter, the estimate used in the base case followed by conservative and liberal estimates. Only one parameter was changed at a time to generate unique benefit-cost ratios for each of the scenarios compared to the base case benefit-cost ratio for universal NBS (1.16).

Table 1. Sensitivity analysis

Parameter	Conservative estimate	Base case	Liberal estimate	Benefit-cost ratio swing
birthrate	69,000	80,000	91,000	1.12 to 1.19
birth prevalence - 1 in:	30000	40000	50000	1.46 to 0.96
% with Wilson disease family hx	0.03	0.08	0.13	1.22 to 1.09
sensitivity	0.921	0.956	0.9999	1.11 to 1.21
specificity	0.981	0.9999	0.9999	0.43 to 1.16
early ID mortality	0.00	0.00	0.05	1.16 to 0.53
% that receive liver transplant	0.05	0.06	0.15	1.14 to 1.25
transplant-related mortality	0.00	0.13	0.26	1.05 to 1.26
late identified mortality	0.017	0.035	0.070	0.96 to 1.55
cost of lifetime zinc treatment (early id)	0.00	146,697.89	293,395.78	1.53 to 0.88
cost of liver transplant	$508,900.00	$1,017,800.00	$2,035,600.00	1.14 to 1.18
value of a life	$6,176,087.05	$13,234,472.25	$20,145,807.76	0.88 to 1.43
value of averted liver transplant	$136,840.51	$273,681.02	$547,362.04	1.15 to 1.17
cost of NBS test	$14.17	$18.89	$23.62	1.46 to 0.96
cost of false (+)	$1,000.00	$2,000.00	$10,000.00	1.16 to 1.14
% w/ neurologic sx	0.161	0.322	0.644	0.83 to 1.81
value of lifetime severe disability	$1,168,986.79	$2,337,973.58	$4,675,947.16	0.83 to 1.81
treatment adherence	60%	100%	100%	0.66 to 1.16

Overall, the base model appears robust in that most of the high or low estimates for the parameters did not affect the benefit-cost ratio to a large degree. Specifically, the ratio never decreases to a negative number, nor does it increase to as much as two.

The biggest changes were for the low specificity estimate, if early identification doesn't completely prevent disability and if WD patients don't adhere to treatment (yellow highlighted cells in Table 1.)

Conclusion

Early identification and treatment of babies with Wilson disease is critical to prevent morbidity and mortality. With universal screening and treatment of asymptomatic individuals, the following benefits are estimated: reduced number of deaths, reduced number of liver transplants, and reduced number of cases with neurologic symptoms.

This analysis used data from primary literature, a local pilot study, and expert opinion to quantify estimated benefits and costs for newborn screening of Wilson disease. Using our best estimates for parameters, the benefit-cost ratio for universal screening was 1.16. For every dollar of costs to provide Wilson disease screening, we predict that there will be $1.16 worth of benefit from universal screening. The annual net benefit from universal screening is $293,448.37. The sensitivity analysis showed that the model is robust because the benefit-cost ratio did not change substantially when more conservative or liberal estimates for parameters were made in the model.

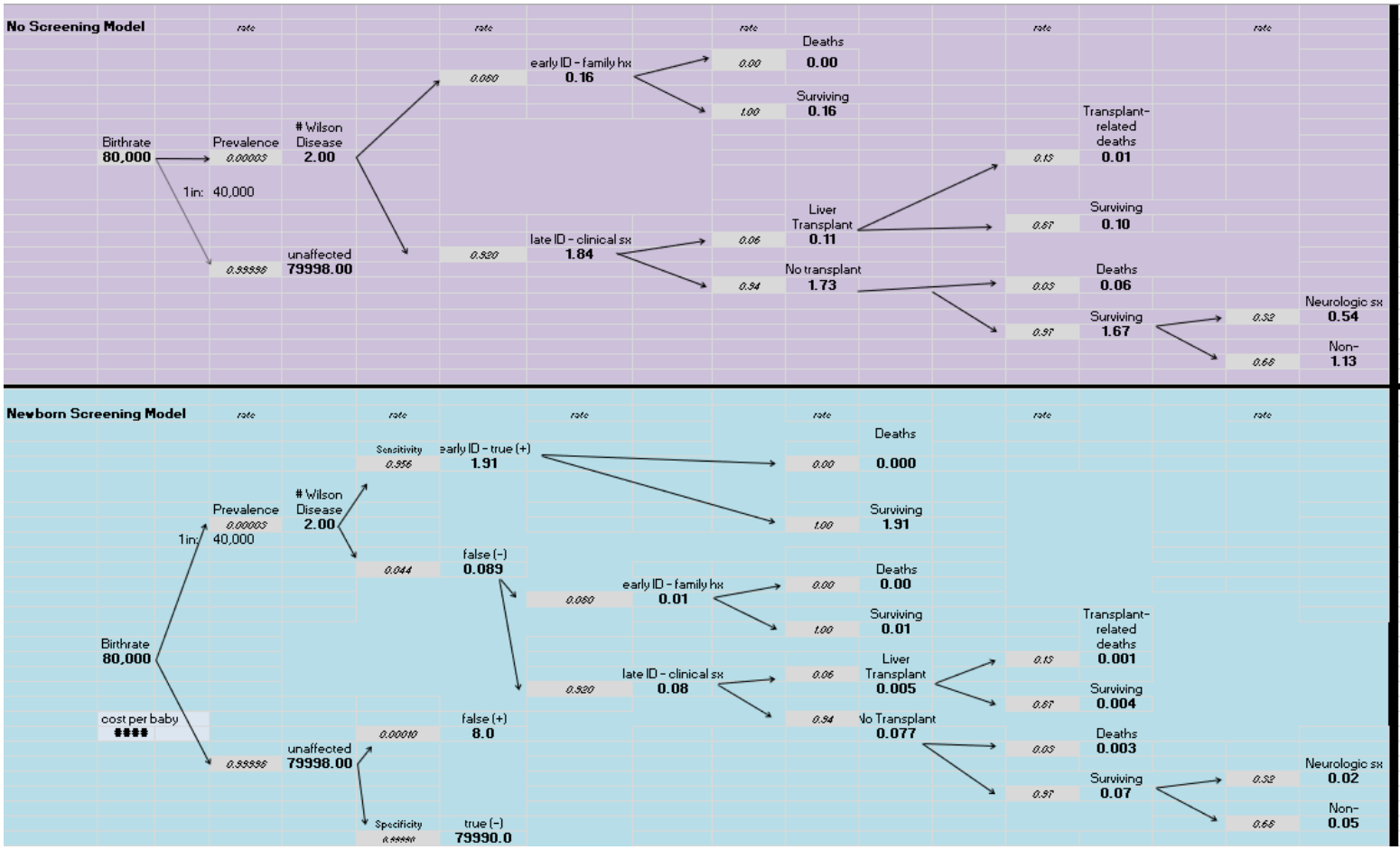
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Figure 1. Washington State Benefit-Cost Analysis for potentially adding NBS for Wilson disease



No Screening	Deaths		0.0749
	Surviving		1.93
	Liver Transplants		0.1140
	Lifetime zinc treatment	$	175,055.47
	Zinc treatment and early mortality	$	2,386.99
	Liver transplant w/ mortality	$	7,590.63
	Liver transplant and surviving	$	54,260.03
	Total tx costs		#########
	Lifetime disability due to neurologic sx	$	1,253,468.07
Lifetime discounted treatment costs:			
	Zinc treatment (late id)		$90,962.76
	Zinc treatment (early id)		$146,697.89
	Zinc treatment and early mortality		$39,731.71
	Liver transplant w/ mortality		$513,061.36
	Liver transplant and surviving		$546,808.16
Annual cost of severe disability associated with neurologic sx:		$	150,000.00
Estimated lifetime cost of severe disability			$2,337,973.58

Screening	Deaths		0.003
	Surviving		1.997
	Liver transplants		0.005
Costs	Lifetime zinc treatment		$288,147.17
	Zinc treatment and early mortality		$105.87
	Liver transplant w/ mortality		$336.66
	Liver transplant and surviving		$2,406.53
	Total tx costs		**$290,996.23**
	Lifetime disability due to neurolic sx		$55,593.63
	costs of screening (ongoing)	$	1,511,479.04
	cost of screening (start up)		$31,411.62
	cost of false (+) dx test per baby	$	2,000.00
	total cost of false(+) dx testing	$	**15,986.81**
	TOTAL costs		#########
SHIFT			
Benefits	Deaths averted		0.0716
	Value of a life		$13,234,472.25
	Value of lives saved		$948,950.965
	Liver transplants averted		0.1090
	Value of liver transplants averted		18,788.16
	Value of severe disability averted		1,197,874.44
	Less treatment costs		-51,703.11
	TOTAL Benefits		$2,111,910.45
	Benefit/Cost ratio		1.16
	net benefit		$293,448.37



Washington State Department of HEALTH

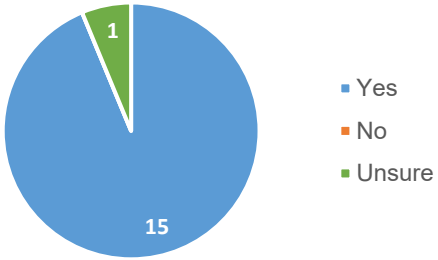
WASHINGTON STATE BOARD OF HEALTH

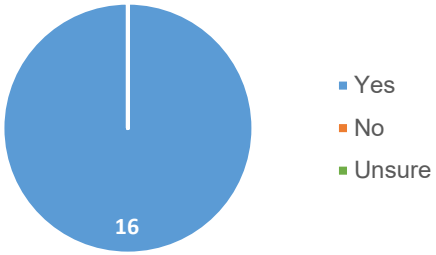
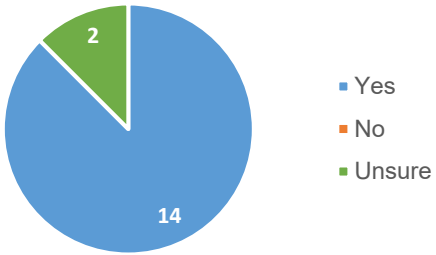
Newborn Screening Technical Advisory Committee (TAC)

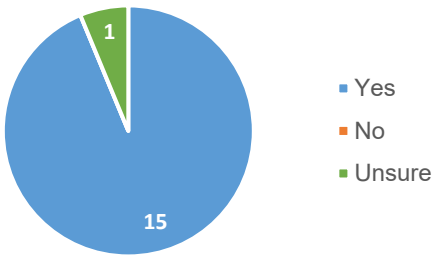
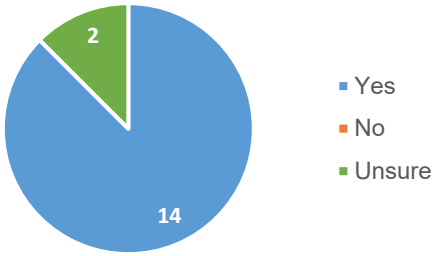
Meeting to Review Wilson Disease for the Newborn Screening Panel

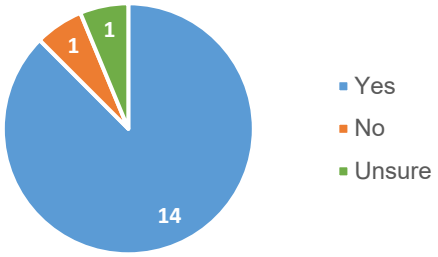
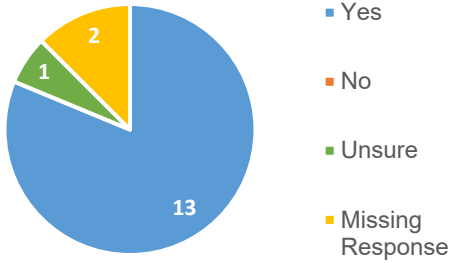
TAC Member Voting Summaries and Comments (n=16)

The following is a compilation of comments from TAC members provided when voting on each individual criteria and an overall recommendation. Comments have been summarized and are organized by each criterion and then overall comments provided.

Criteria	Comments or Major themes
1. Available Screening Technology 	• One commenter was uncertain about the peer review of Wilson Disease screening due to limited studies though noted the research is specific to the state and includes diverse ethnic populations.

2. Diagnostic Testing and Treatment Available 	• N/A
3. Prevention Potential and Medical Rational 	• Five members acknowledged that Wilson disease may not present clinically within the first year of life (as specified in the criterion) but emphasized that the disease process begins at birth. They supported screening in the newborn period based on this early onset of pathology. • Several members suggested revisiting the criterion language, such as changing "must" to "should" to better reflect the intent.

4. Public Health Rationale 	• N/A
5. Cost Benefit-Cost Effectiveness 	• N/A

6. Public Health Infrastructure Readiness 	• One member expressed concern that Wilson Disease may not meet the readiness criteria. • Another member appreciated that the Seattle Children's Wilson Disease Center of Excellence has capacity to accept new patients and that the condition can be managed remotely.
Overall Recommendation 	• Several TAC members expressed strong support for adding Wilson Disease to the newborn screening panel. • One member highlighted the availability of a highly sensitive and specific assay, a state-coordinated population-based screening process, low-cost interventions, and effective treatments that support normal development. • Another emphasized that, even if all criteria are not fully met, the potential health benefits and importance of early detection justify its consideration. • One commenter suggested that if current funding constraints delay implementation, the Board should still approve the condition for inclusion once resources become available.

PETITION FOR ADOPTION, AMENDMENT, OR REPEAL OF A STATE ADMINISTRATIVE RULE

Print Form

In accordance with RCW 34.05.330, the Office of Financial Management (OFM) created this form for individuals or groups who wish to petition a state agency or institution of higher education to adopt, amend, or repeal an administrative rule. You may use this form to submit your request. You also may contact agencies using other formats, such as a letter or email.

The agency or institution will give full consideration to your petition and will respond to you within 60 days of receiving your petition. For more information on the rule petition process, see Chapter 82-05 of the Washington Administrative Code (WAC) at http://apps.leg.wa.gov/wac/default.aspx?cite=82-05.

CONTACT INFORMATION *(please type or print)*

Petitioner's Name: Dr. Sihoun Hahn, M.D, Ph.D.

Name of Organization: Key Proteo, Inc.

Mailing Address: 720 Broadway

City: Seattle State: WA Zip Code: 98122

Telephone: 206-399-7515 Email: steve.hahn@keyproteo.com

COMPLETING AND SENDING PETITION FORM

- Check all of the boxes that apply.
- Provide relevant examples.
- Include suggested language for a rule, if possible.
- Attach additional pages, if needed.
- Send your petition to the agency with authority to adopt or administer the rule. Here is a list of agencies and their rules coordinators: http://www.leg.wa.gov/CodeReviser/Documents/RClist.htm.

INFORMATION ON RULE PETITION

Agency responsible for adopting or administering the rule: Washington State Board of Health

☒ **1. NEW RULE - I am requesting the agency to adopt a new rule.**

☒ The subject (or purpose) of this rule is: Institute newborn screening of Wilson Disease for Washington State.

☒ The rule is needed because: NBS and early diagnosis for Wilson Disease is currently an unmet need.

☒ The new rule would affect the following people or groups: Newborns with Wilson Disease; Parents and families negatively impacted by association.

☐ **2. AMEND RULE - I am requesting the agency to change an existing rule.**

List rule number (WAC), if known: _____

☐ I am requesting the following change: _____

☐ This change is needed because: _____

☐ The effect of this rule change will be: _____

☐ The rule is not clearly or simply stated: _____

☐ **3. REPEAL RULE - I am requesting the agency to eliminate an existing rule.**

List rule number (WAC), if known: _____

(Check one or more boxes)

☐ It does not do what it was intended to do.

☐ It is no longer needed because: _____

☐ It imposes unreasonable costs: _____

☐ The agency has no authority to make this rule: _____

☐ It is applied differently to public and private parties: _____

☐ It conflicts with another federal, state, or local law or rule. List conflicting law or rule, if known: _____

☐ It duplicates another federal, state or local law or rule. List duplicate law or rule, if known: _____

☐ Other (please explain): _____

[Supplemental Documents]

1. Criteria Justification
2. Petition Letter
3. WA State Pilot Study Overview Slide deck
4. NBS Kit validation for FDA submission Slide deck
    a. IFU
    b. Analytical & Clinical Validation data for FDA submission
5. References
    a. APHL abstract 2024
    b. Ann. N.Y. Acad. Sci: Population screening for Wilson disease
    c. Clinical and Translational Perspectives on Wilson disease. Edited by Nanda Kerkar and Eve A. Roberts
        i. Chapter 26. Population screening for Wilson disease
        ii. Chapter 17. Wilson disease in Infancy through Adolescence
    d. Handbook of Clinical Neurology. Wilson Disease Edited by Anna Czlonkowska and Michael L. SChilsky
        i. Chapter 3.The genetics of Wilson Disease
    e. J. Proteome Res. 2017: Quantification of ATP7B Protein in Dried Blood Spots by Peptide Immuno-SRM as a Potential Screen for Wilson's Disease
    f. Gastroenterology 2021: Direct Measurement of ATP7B Peptides Is Highly Effective in the Diagnosis of Wilson Disease
        i. Editorial comments: Expanding the Diagnostic Toolkit of Wilson Disease with ATP7B Peptides
    g. JPGN 2020: Management of Wilson Disease Diagnosed in Infancy: An Appraisal of Available Experience to Generate Discussion
    h. GeneReviews: Wilson disease: Wilson Disease - GeneReviews® - NCBI Bookshelf (nih.gov)
6. Letter of Support
    a. Wilson Disease Association, U.S.A.
    b. Wilson Disease Association, Spain
    c. Experts
        i. Dr. Karl Weiss in Germany
        ii. Dr. Michael Schilsky at Yale University
    d. Family members:
        i. Letters from US

- Alice William Family
- Christopher and Rachel Johnson
- Nora Closser
- Erin Brooks
- Dr. Kirk Vestal
- Thomas Sandall
- Marilee and Gary Wolter
- Maxine and Michael Bonn
- Janet Laubgross

ii. Letter from Poland

- Anna Aniol

THREE GUIDING PRINCIPLES: Three guiding principles govern all aspects of the evaluation of a candidate condition for possible inclusion in the NBS panel.
• Decision to add a screening test should be driven by evidence. For example, test reliability and available treatment have been scientifically evaluated, and those treatments can improve health outcomes for affected children.
• All children who screen positive should have reasonable access to diagnostic and treatment services.
• Benefits of screening for the disease/condition should outweigh harm to families, children and society

[Criteria for Testing]

Available Screening Technology: Sensitive, specific and timely tests are available that can be adapted to mass screening.

The base technology is tandem mass spectrometry (LC-MS/MS), which is already being used for NBS conditions such as LSD (Lysosomal Storage Disorders) and XLD (X-linked Adrenoleukodystrophy) in Washington State. Quantification of ATP7B protein using LC-MS/MS utilizes trypsin to digest protein and quantify the resulting signature surrogate peptides of the target protein, ATP7B. It has been well demonstrated that the assay can provide the diagnosis and newborn screening of Wilson disease with sensitivity and specificity both above 92%. To date, over 25,000 newborns have been successfully tested in Washington State. A total of 4 presumptive positive cases have been detected. Two of them were false positive (no variants or carriers) and two of them were likely true positive. The sample to sample run time is less than 3 minutes which is appropriate for high throughput analysis.

Diagnostic Testing and Treatment available: Accurate diagnostic tests, medical expertise, and effective treatment are available for evaluation and care of all infants identified with the condition.

Biochemical and genetic testing are widely available to confirm the diagnosis. Treatment includes a copper restricted diet, zinc or chelating agents such as penicillamin or syprine. Gene therapy is currently under clinical trial. Liver transplantation is required in advanced patients with decompensated liver cirrhosis and acute liver failure. The cost for the maintenance treatment with zinc is a penny a day ($.01/day).

Prevention Potential and Medical Rationale: The newborn identification of the condition allows for early diagnosis and intervention. Important considerations:

• *There is sufficient time between birth and onset of irreversible harm to allow for diagnosis and intervention.*
The newborn identification of the condition allows for early diagnosis and preemptive intervention. There has been a strong need for NBS of Wilson disease (WD), which progresses to an irreversible multi-systemic disease. The patient mortality rate is 100% if left untreated. The copper accumulation starts right after birth but as the disease progress is slow, WD is usually diagnosed in adolescents after significant complications occur. Nonetheless, it is now well established that Wilson disease can present with clinical disease, mainly hepatic, in very young children <5 years old. Since the first detailed and well-documented report of a 3-year-old with cirrhosis, numerous reports have appeared providing additional evidence to institute NBS for Wilson disease.

Unlike other metabolic disorders, infants with Wilson disease do not present with any acute deterioration in the first few years of life. It is generally recommended to start the zinc maintenance

treatment during 12-24 months of life and monitor the labs every 6 months or a year. There is ample time to allow for diagnosis, counseling and initiation of treatment.

• *The benefits of detecting and treating early onset forms of the condition (within one year of life) balance the impact of detecting late onset forms of the condition.*
The symptoms of Wilson disease are frequently nonspecific. Approximately one-third of children with clinically evident WD are present with cirrhosis. Acute liver failure poses significant diagnostic problems. Although neurologic Wilson disease is less common in pediatric patients with Wilson disease, it has been reported in children as young as 6 years old. Initial neurological symptoms could be extremely nonspecific further delaying the diagnosis and appropriate management. Treatment for neurological Wilson disease is challenging as they could develop further deterioration with chelating agents.

The cost for zinc maintenance treatment is only a penny a day ($.01/day) when started before any complications occurred and the patients live a completely normal life. The benefits of detecting and treating the condition before symptoms occurred have been well described in many literatures.

• Newborn screening is not appropriate for conditions that only present in adulthood.
As many genetic disorders have a spectrum of clinical severity, some patients with Wilson disease may not have the symptoms until later in life. However, all those biochemically and genetically confirmed Wilson disease patients should start the treatment regardless of the symptoms or lab results at the time of the diagnosis.

Public Health Rationale: Nature of the condition justifies population-based screening rather than risk-based screening of other approaches.

Effective treatment for Wilson disease is widely available and affordable, highlighting the importance of early detection and intervention. These treatments are highly effective in preventing the downstream of morbidity and early mortality associated with Wilson disease. While there are effective treatments available, it is unfortunate that most patients come to the clinic after developing serious complications. Furthermore, many patients suffer from delayed diagnosis due to the nature of nonspecific and considerably variable clinical presentations. Half of the affected patients with neurological symptoms may develop permanent neurological damage. Patients with chronic liver disease may progress to cirrhosis and hepatocellular carcinoma.

Importantly, patients with Wilson disease can live a completely normal life when the treatment is started early in life. Early initiation of therapy before the onset of symptoms makes a critical difference in their outcome. The cost for zinc treatment is nominal compared to other cell based or gene therapies. The best clinical outcome could be achieved only through NBS.

Cost-benefit/Cost-effectiveness: The outcome outweighs the costs of screening. All outcomes, both positive and negative, need to be considered in the analysis. Important considerations to be included in economic analyses include:

Newborn screening of Wilson disease will change the diagnostic course and clinical outcome of WD patients. As many WD patients undergo liver transplantation, this opportunity for early initiation of therapy before the onset of symptoms will significantly make a critical and huge difference in their long-term clinical outcome. Ultimately, we anticipate there will be savings that come from converting patients who would otherwise be consumers of high-intensity medical care, long-term convalescent care, and disability resources, to functional individuals capable of holding jobs and contributing to the tax base.

- *The prevalence of the condition among newborns.*

The prevalence of WD is approximately 1 in 30,000 newborns, with a carrier frequency of 1 in 90 (higher in certain populations). However, regional variations exist. Costa Rica, Sardinia, the Canary Island and Crete have all reported to have increased incidence.

• *The positive and negative predictive values of the screening and diagnostic tests.*

ATP7B 887 analysis was found to have a sensitivity of 91.2%, specificity of 98.1%, positive predictive value of 98.0%, and a negative predictive value of 91.5%. ATP7B 1056 showed a positive predictive value of 96.1% and negative predictive value of 91.3%.

• *Variability of clinical presentation by those who have the condition.*

Wilson disease presents a variety of symptoms depending on which organs are more affected. Wilson disease can lead to hepatic failure and/or severe neurological disability and ultimately death if untreated.

• *The impact of ambiguous results. For example, the emotional and economic impact on the family and medical system.*

The current pilot study in Washington State indicates the false positive rate is extremely low compared to the other conventional newborn screening methods.

• *Adverse effects or unintended consequences of screening.*

As with other screening programs, there could be false negative cases from this screening.

720 Broadway
Seattle, WA 98122—4302
206-339-7515
KEY PROTEO

To: Washington State Department of Health
Board of Directors

From: Sihoun Hahn, MD, PhD
Professor of Pediatrics, University of Washington
Seattle Children's Hospital
Founder and CMO, Key Proteo, Inc

Re: Letter from Petitioner

Date: July 26, 2024

Dear Washington State Department of Health Board of Directors,

On behalf of Key Proteo, I am writing to request your review of our Petition for Rulemaking to adopt a new administrative rule to screen for Wilson Disease (WD). Within our petition package you will find evidence to support Washington States Qualifying Assumption, evidence to support the Three Guiding Principles, as well as evidence to support the 5 Criteria used to evaluate conditions for possible inclusion in the newborn screening panel.

Wilson Disease is an autosomal recessive disease of copper metabolism caused by pathogenic mutations in the *ATP7B* gene, a copper-transporting ATPase. About 1 out of 30,000 individuals are affected by WD. Early diagnosis and pre-emptive therapy before the manifestation of symptoms are crucial for successful clinical outcomes that can lead to a completely normal life with a cost of a penny a day ($.01/day) with zinc treatment. Without early intervention, individuals can develop significant life-threatening complications, including liver cirrhosis, acute kidney failure, and brain and nerve damage. Key Proteo's Immuno-Selected Reaction Monitoring (SRM) platform is a novel proteomic screening solution that uniquely delivers rapid, scalable, and cost-effective detection of extremely low abundance peptide biomarkers in newborn dried blood spot specimens with high accuracy, effectiveness, and efficiency via familiar mass spectrometry workflows currently being used in the Washington State Newborn Screening Laboratory. By quantifying the ATP7B peptide, Key Proteo's technology provides accurate results to identify affected patients allowing them to be treated clinically leading to healthier outcomes. We have been successfully running a pilot study in collaboration with WA State DOH Newborn Screening Laboratory (Director Dr. John Thompson) for over two years screening ~25,000 newborn babies for Wilson disease. The progress report is included in the supplemental package.

Our petition package includes letters of support from the Wilson Disease Association (US), Wilson Disease Association (Spain), experts in the field such as Drs. Karl Heinz Weiss and Michael Schilsky, and families from the U.S., and Poland providing additional support as well as sharing their stories. We've also included evidence to support the need to adopt this test including validation data, Instructions for Use, criteria justification, as well as literature references.

It is our immense hope that Washington State will adopt and require this test for all newborns screened in the newborn screening program which will be the first model to all other states and the world. Together we can provide the community with early diagnosis, healthier outcomes, and relieve the heavy

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KEY PROTEO

burden these families carry when confronted with unexpected but life-saving decisions due to late diagnosis.

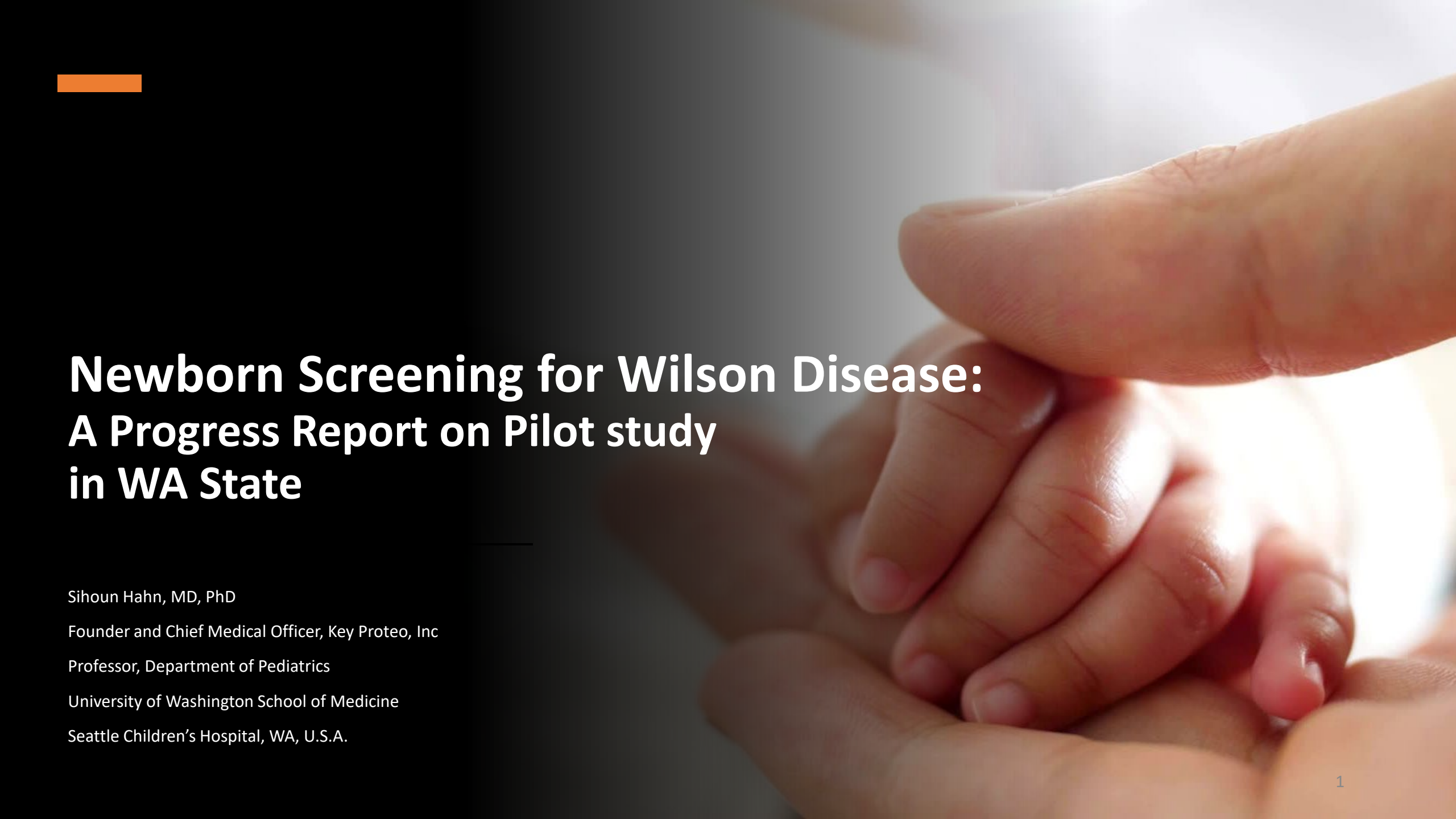
We appreciate your consideration and look forward to your review. Please do not hesitate to reach out with any questions whatsoever.

Sincerely,

Sihoun Hahn Verified by pdfFiller
07/26/2024

Sihoun Hahn, MD, PhD
Director, Center of Excellence for Wilson disease
Seattle Children's Hospital
Founder and CMO, Key Proteo, Inc

Newborn Screening for Wilson Disease:
A Progress Report on Pilot study in WA State



Sihoun Hahn, MD, PhD

Founder and Chief Medical Officer, Key Proteo, Inc

Professor, Department of Pediatrics

University of Washington School of Medicine

Seattle Children's Hospital, WA, U.S.A.

FINANCIAL DISCLOSURES

Sihoun Hahn, MD, PhD, is a member of the Seattle Children's Hospital workforce and is serving as Chief Medical Officer of Key Proteo, Inc. He is an inventor of intellectual property that has been licensed to Key Proteo, Inc. Dr. Hahn is the founder of Key Proteo, Inc. and has ownership equity interests in the company.

Topics to discuss

- Background of Wilson disease
- Proof of evidence study on a large-scale patient cohort
- Manufactured kit validation for public health transition
- Pilot study in WA state

Background of Wilson Disease

Wilson Disease

- Autosomal Recessive Copper Transport Disorder
- Incidence: ~1/30,000
- Early diagnosis and pre-emptive treatment can lead to a normal quality of life



Dr. Samuel A. K. Wilson (1878-1937)

- Progressive, fatal disease if untreated
- ***ATP7B* gene**: ATPase, copper transporter
  - >1700 variants (Varsome)
  - p.H1069Q : ~35-45% in European origin
  - p.R778L: ~30% in Far East Asian origin





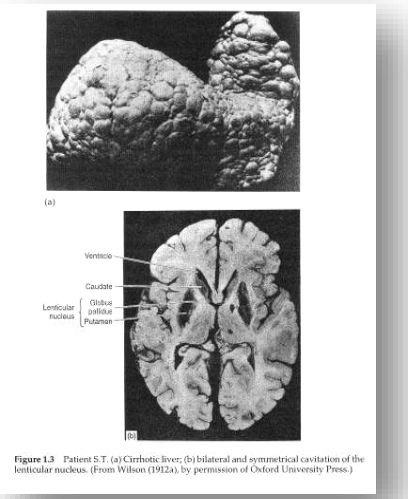


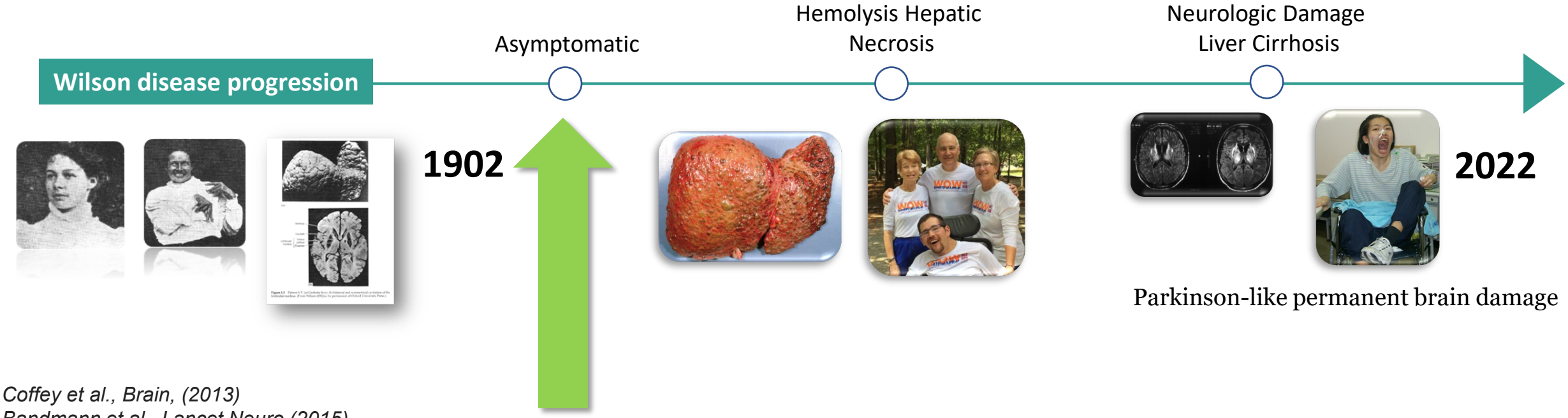
Figure 1.3 Patient S.T. (a) Cirrhotic liver; (b) bilateral and symmetrical cavitation of the lenticular nucleus. (From Wilson (1912a), by permission of Oxford University Press.)

Hoogenraad TU, Wilson's disease, Intermed Medical Publishers

- Diagnosis: high copper in the liver/urine, low serum ceruloplasmin, KF ring in the cornea
- Treatment: Chelators (Trientine), Zinc salts, Gene therapy under trial
- ***Immuno-SRM analysis is promising for NBS***
  - ***Majority of mutations results in markedly decreased level of ATP7B protein due to enhanced degradation, absence or decay of mRNA*** *(Hepatology, 2009; Proc Natl Acad Sci U S A, 1998; Gastroenterology, 2007; Curr Issues Mol Biol, 2001; Proteins, 2008; Genetics, 1998; Blood, 2005; Mol Genet Metab, 2005; BMC Gastroenterol, 2010; Nat Genet, 2004; Annu Rev Biochem, 2007)*

With early intervention, we can change the clinical course

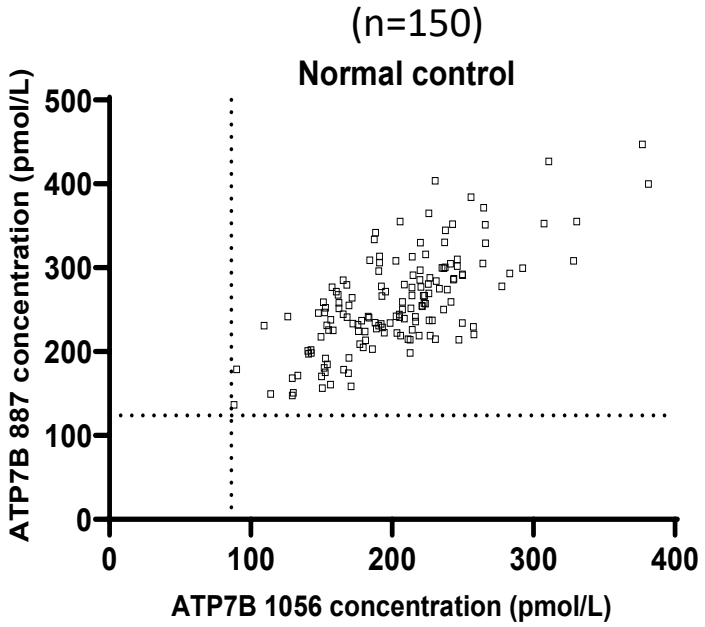
- Unrecognized in a substantial portion of affected individuals [1-2]
  (At least half of patient with WD are **never diagnosed** and die of untreated disease)
- Late diagnosis is the most common cause of death [3]
- Early diagnosis improves clinical outcome [4]
- Currently, there is no screening method while effective treatments are available

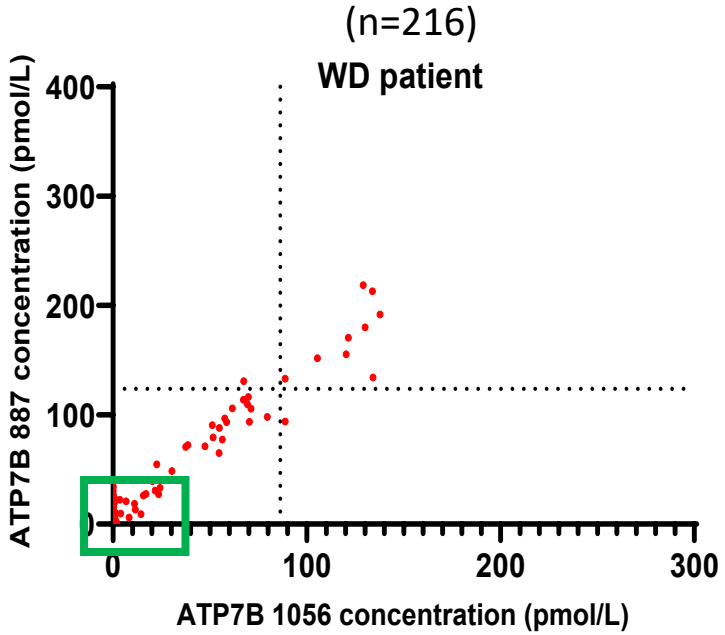


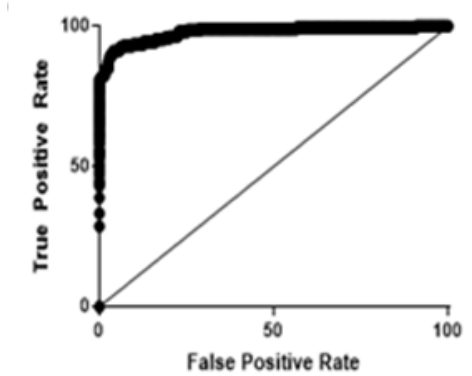
1. *Coffey et al., Brain, (2013)*
2. *Bandmann et al., Lancet Neuro (2015)*
3. *Walshe . Mov Disord. (2007)*
4. *Beinhardt et al, Clini Gastroenterol Hepatol (2014)*

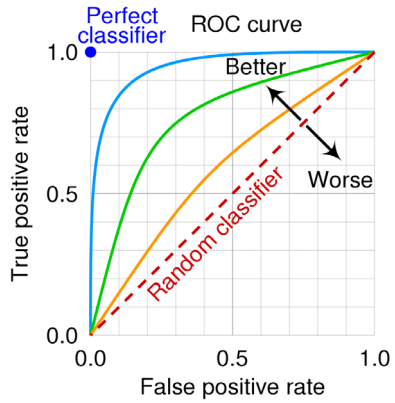
Proof-of-Evidence Study on a Large-Scale Patient Cohort

Wilson Disease: Most patients are deficient in ATP7B peptides







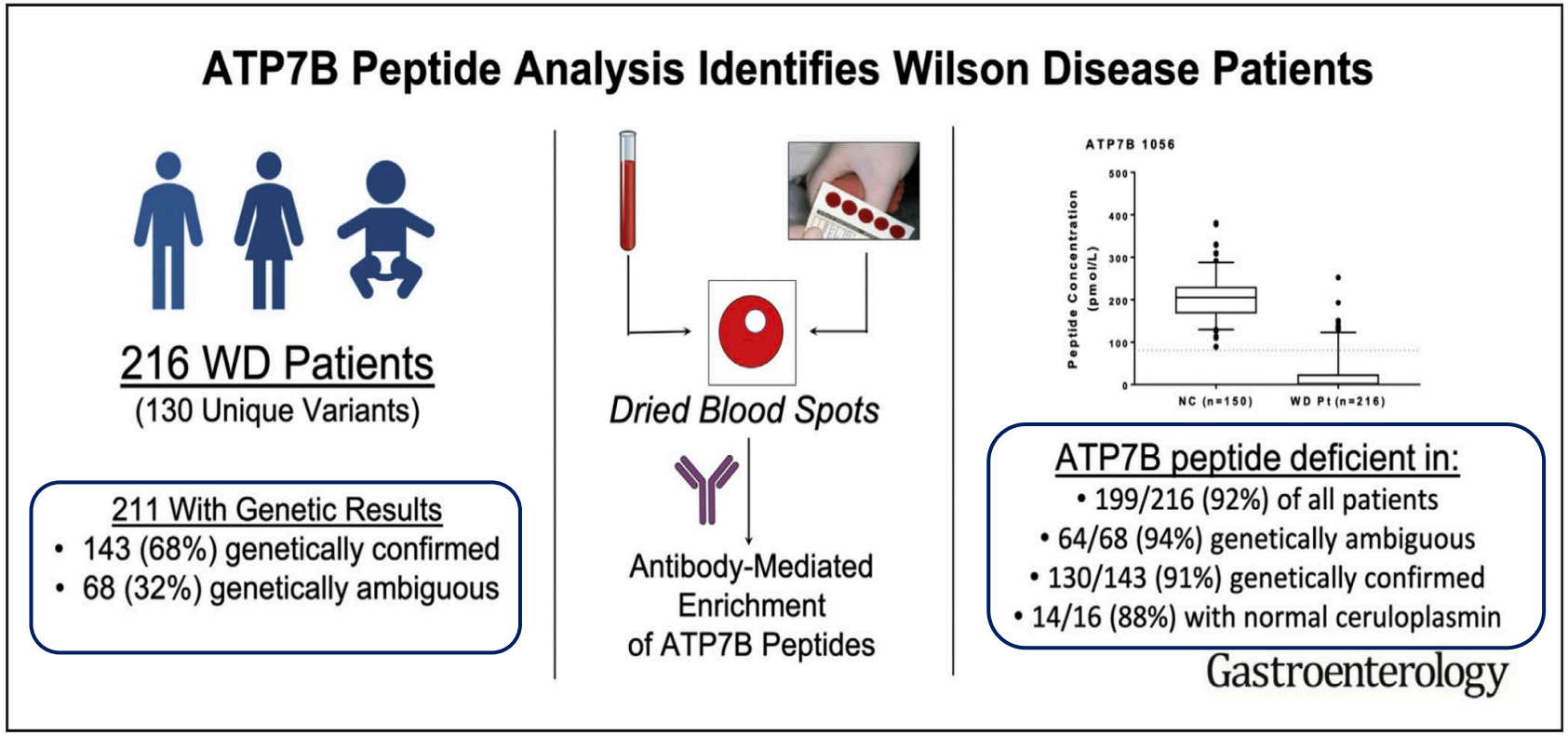


- **Large Retrospective Study**
  - 216 WD patients, 48 carriers,150 healthy controls
  - ATP7B peptides enriched from DBS
- 199 / 216 patients (92.1%) had at least 1 ATP7B peptide below diagnostic cutoff.
  - ~80% of patients had ATP7B < 32 pmol/L and <56 pmol/L for ATP7B 1056 and ATP7B 887 respectively
- ROC curve shows AUC 0.98, sensitivity 91.2%, specificity 98.1%, PPV 98.0% and NPV 91.5%

Collins C.J. et al. Gastroenterology. (2021)

Wilson Disease: Direct measurement of ATP7B peptides reduces clinical ambiguity

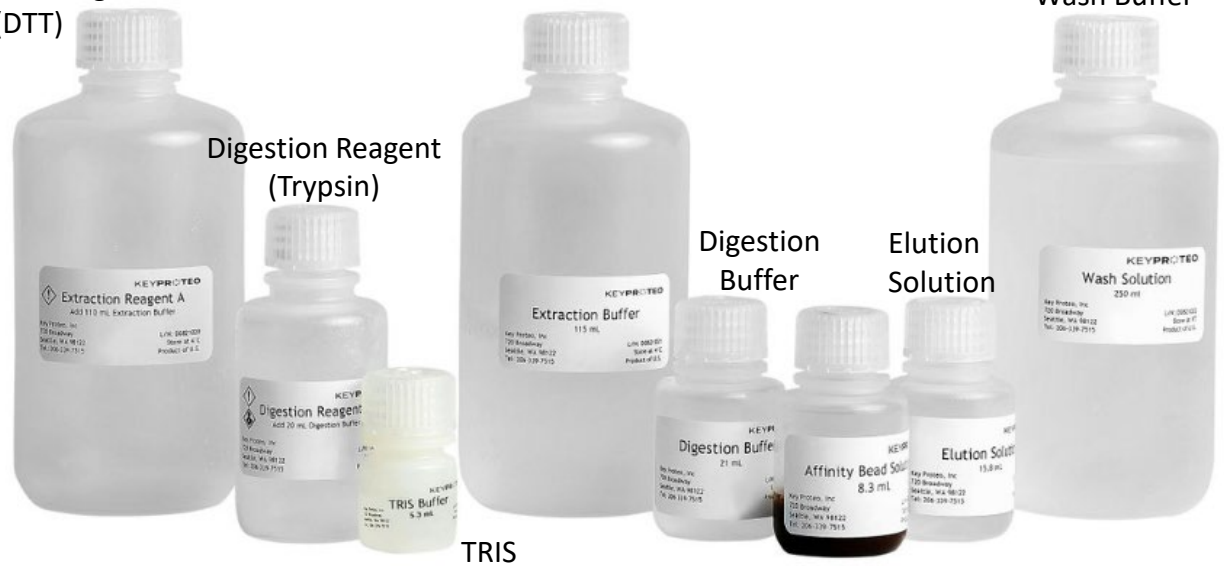
- *Collins C.J. et al. Gastroenterology. 2021, 160(7):2367-2382*
- *Editorial comment. Expanding the Diagnostic Toolkit of Wilson Disease with ATP7B Peptides*



Manufactured Kit Validation for Public Health Transition

Public Health Transition: Prototype 4-plex Screening Reagents

- Prototype reagents for assay performance have been manufactured at GMO facility
- Affinity beads mixed into a single solution
- Internal Standard Peptides lyophilized into 96-well plates
- DBS Control Cards
  - 3 levels of DBS spots
  - Negative, Low, High



Internal Standard Plates

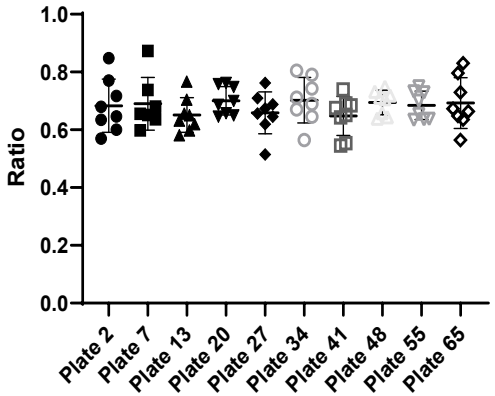


Control Cards

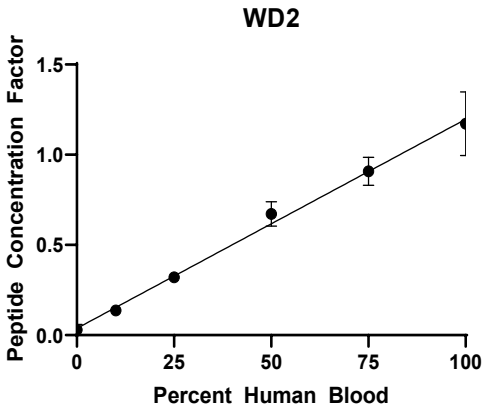


Prototype reagents are not FDA approved and currently intended for investigational use only

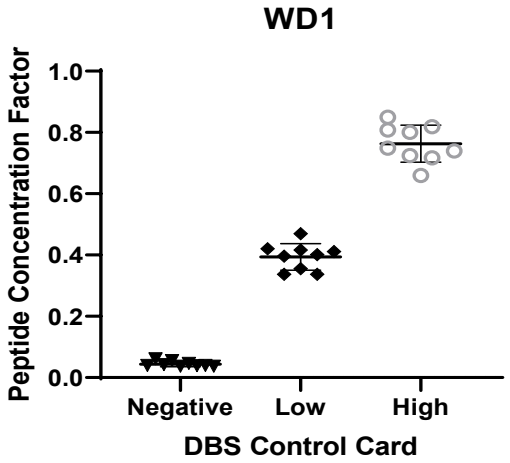
Performance: *Full assay performance and DBS control cards*



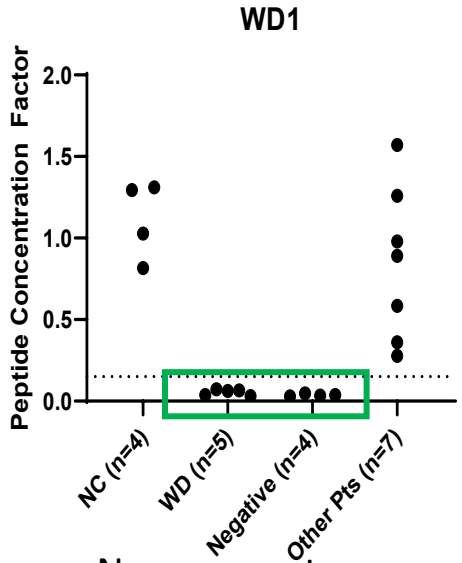
- *IS Plates ensured consistent peptide measurements and maintain consistent cutoff*
  - No dissolution/dilution of IS stock



- *Linear response from decreasing percentage of human blood.*
  - 0, 10, 25, 50, 75 and 100% human



- *DBS control cards perform as expected.*
  - Linear change in measured concentration



- New reagents, workflows, and analysis positively identify blinded patient samples.

Pilot Study in WA State

WA State Pilot Study

In Q1 2022, a large-scale pilot study began in WA State

- In conjunction with the ***WA State Public Health Newborn Screening Laboratory***
- De-identified newborn DBS collected through routine course

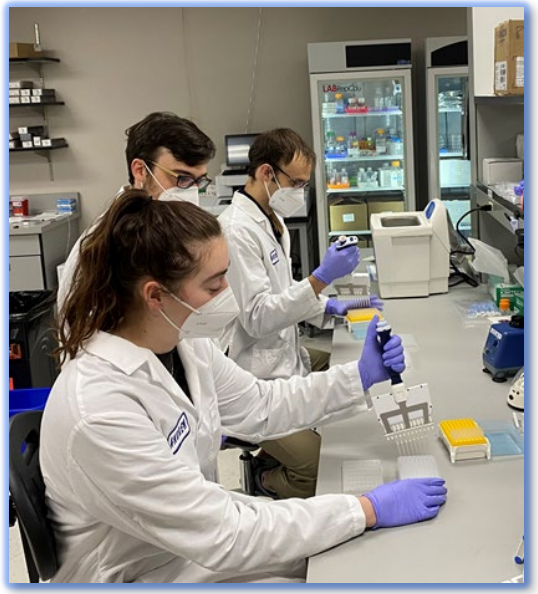
Multiplexed screening utilizing the Neo-WA kit reagents

- Screening for WD, WAS, XLA, and ADA Deficiency
- 50,000-75,000 newborn samples targeted

Samples below initial cut-offs are sequenced for confirmation



WA PHL beginning KP Pilot



Analyzing KP Pilot Samples

WA Pilot Study of Newborn Screening:
Cut-off as of 7/2024

- Initially, the ionKey Microfluidic Separation Chromatography was used, which was later changed to more affordable low-flow ESI probe assembly fitted with a narrow bore capillary suitable for use with flow rates from 5 µL/min to 100 µL/min

- *Ionkey (plates 1-166)*

	ATP7B 887	ATP7B 1056
MEDIAN	359.8	292.4
SD	149.7	99.2
CV	41.6	33.9
Number	14,607	14,607
CUTOFF	68.4	55.5

- *ESI Low Flow (plates 179-current)*

	ATP7B 887	ATP7B 1056
MEDIAN	272.0	270.2
SD	98.1	90.3
CV	36.1	33.4
Number	10,139	10,141
CUTOFF	76.2	75.7

*cutoff is tentative as for now

WA Pilot Study of Newborn Screening: *Demographics*

- A total of ~25,000 newborns have been analyzed to date (7/2024).

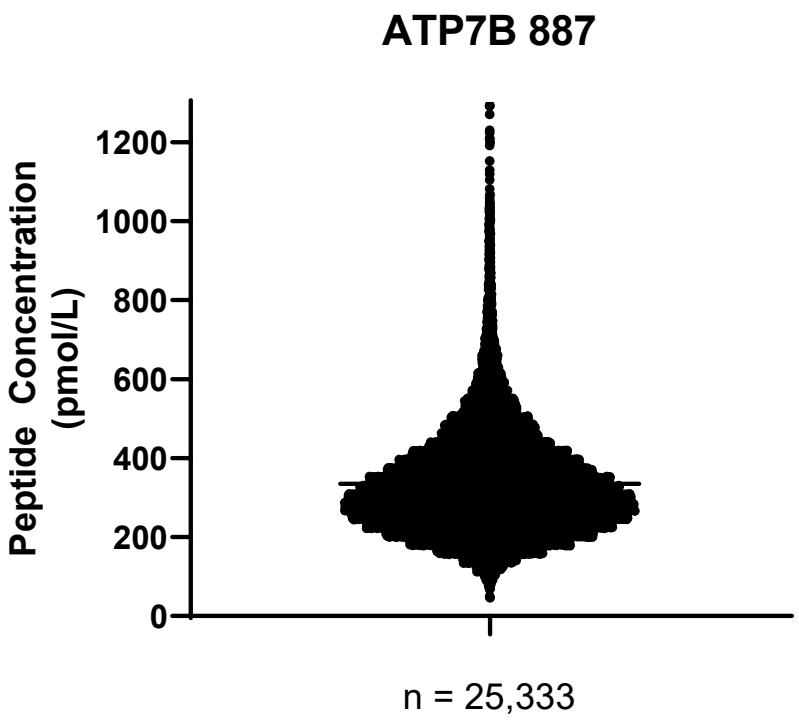
Category	Number
Male	13,035
Female	12,298
< 1500 g BW	258
1500 - 2500 g BW	1,533
2501 – 3500 g BW	13,995
> 3500 g BW	9,547

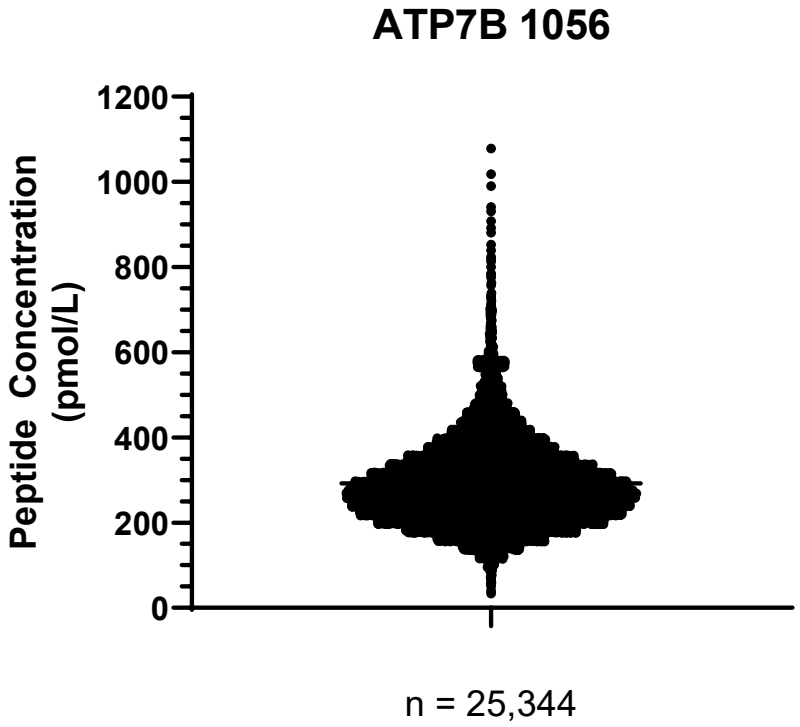
DOC After Birth	Number
0 day (< 24 hours)	4,044
1 day	10,345
2 day	5,608
3 day	3,285
4 day	1,165
5 day	479
6 - 14 days	407

Ethnicity	Number
White	15,603
Hispanic	4,967
Asian	2,813
Black	1,803
Native American	518
Other	910

WA Pilot Study of Newborn Screening: *Peptide Conc.*

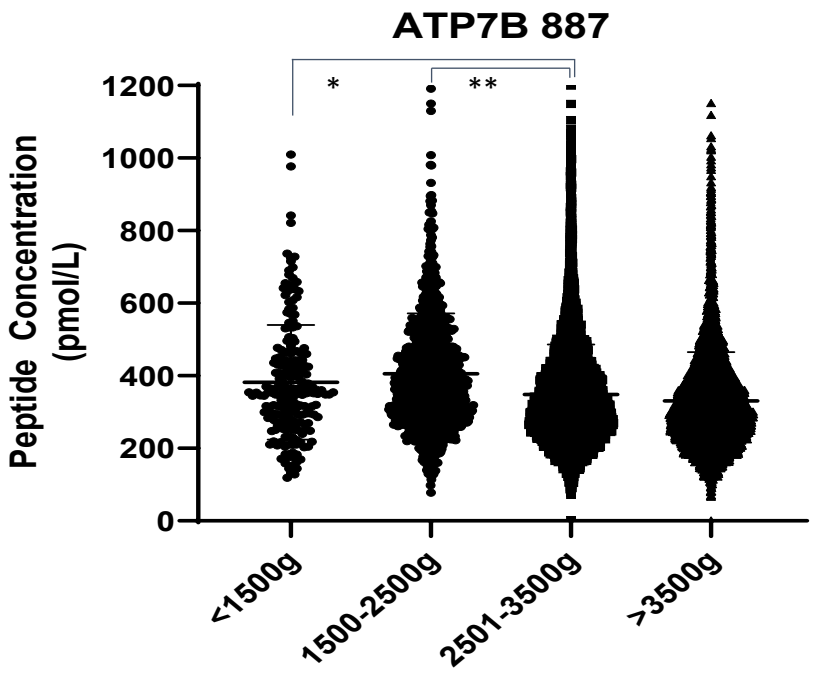
- *Two peptide concentration distributions were identical*

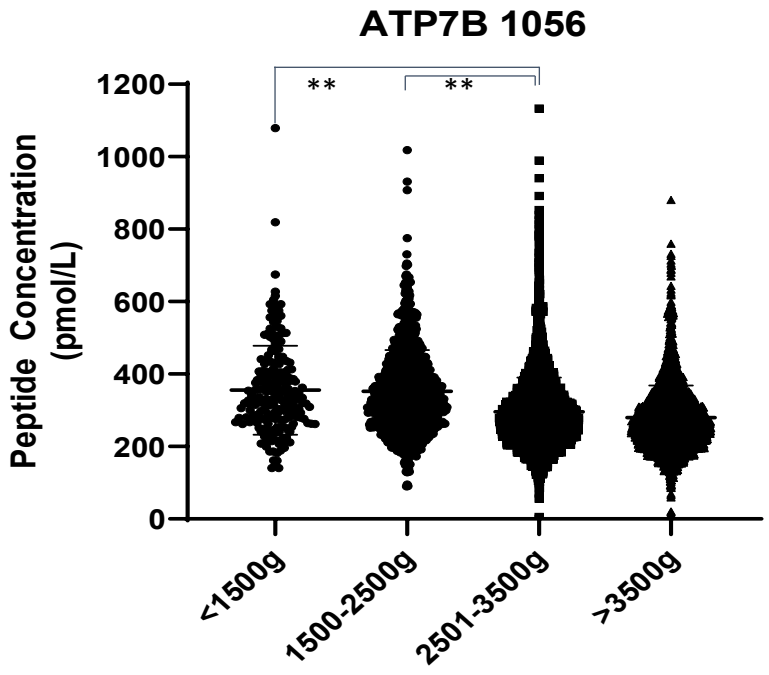




WA Pilot Study of Newborn Screening : *Birth Weight*

- *Peptide concentration was slightly higher in low birth weight but did not impact the overall cutoff range*





*P<0.01
**p<0.001

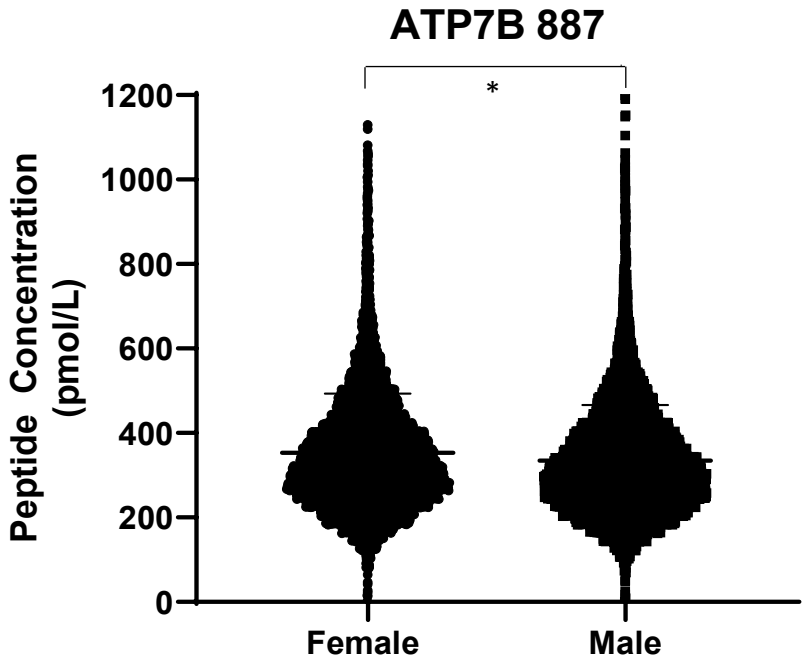
WA Pilot Study of Newborn Screening : *Birth Weight*

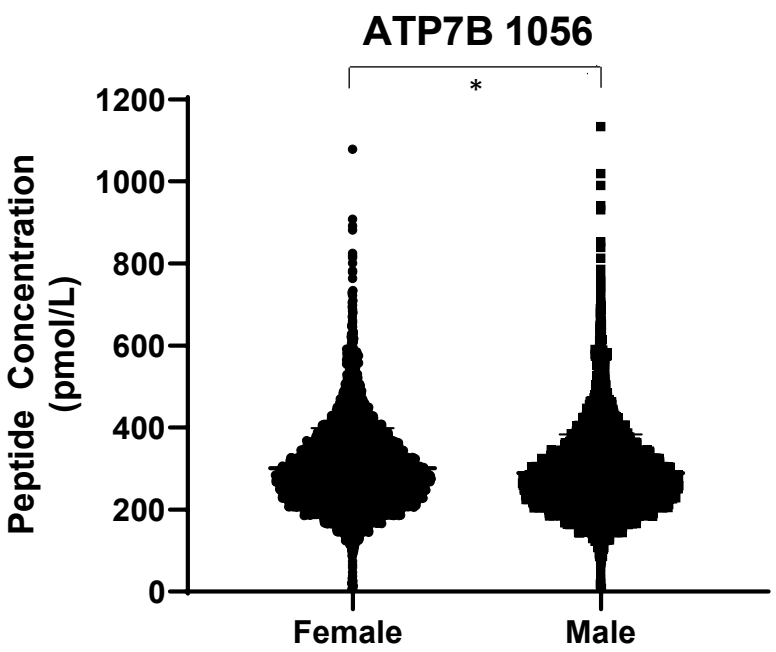
Statistical Analysis

Peptide	<1500g mean (pmol/L)	1500-2500g mean (pmol/L)	>2500g Mean (pmol/L)	p-val <1500g vs >2500g	p-val 1500-2500g vs. >2500g
887	382.0	405.6	341.6	0.0008	<0.0001
1056	355.3	352.4	289.6	<0.0001	<0.0001

WA Pilot Study of Newborn Screening : *Gender*

- *Difference between gender is minimal though statistically significant due to high number of samples tested*





*P<0.001

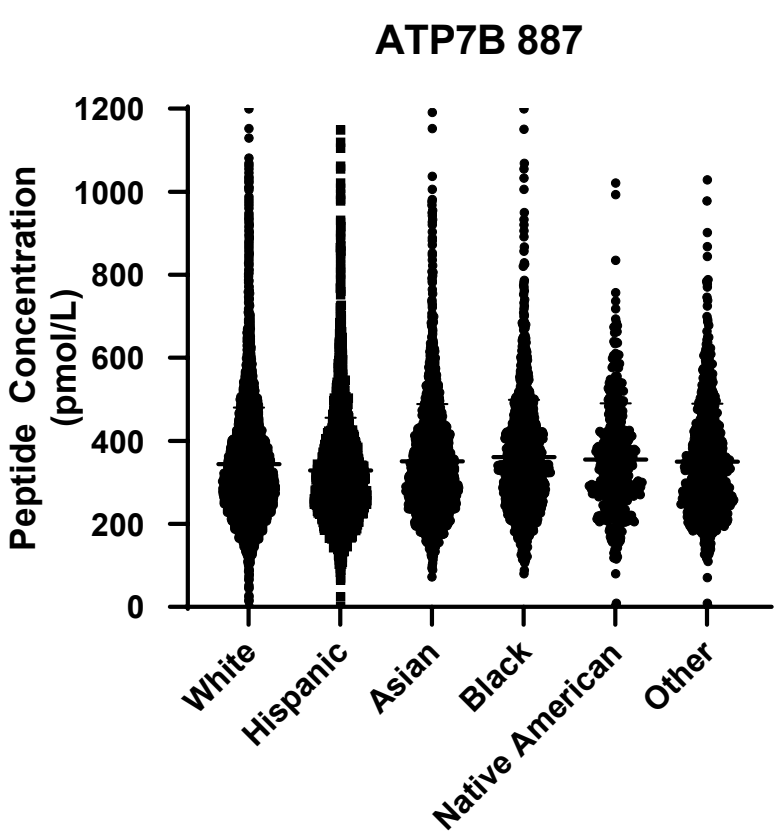
WA Pilot Study of Newborn Screening : *Gender*

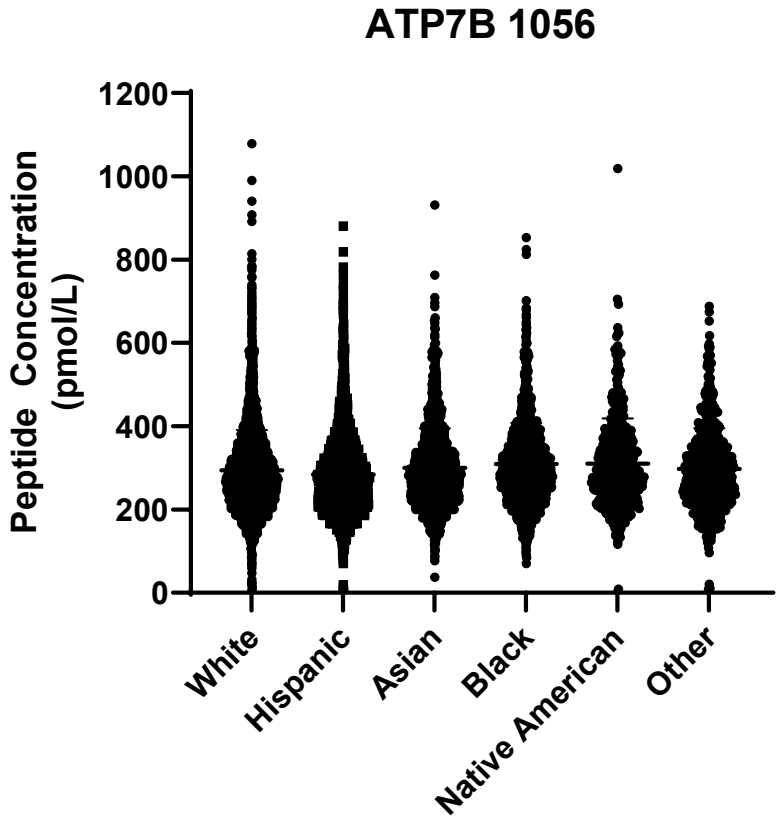
Statistical Analysis

	Female mean (pmol/L)	Male mean (pmol/L)	Difference between means	p-value
887	352.2	334.4	18.4	<0.0001
1056	288.7	301.1	12.4	<0.0001

WA Pilot Study of Newborn Screening : *Ethnicity*

- *No difference in various ethnicities*





Four presumptive positive cases detected

- Please note that current cut-off was tentatively set which can be readjusted in the future
- Two False positive (2/25,000 = 0.008%)
- One likely true positive with two VUS
- One likely true positive DNA pending

	BW (g)	Sex	ATP7B 887 (pmol/L)	ATP7B 1056 (pmol/L)	Genotype	Note
1	3690	M	67.6	134.5	c.3402del/no second variant	carrier
2	4105	M	71.0	96.5	p.Pro610Leu/p.Arg1224Leu	Presumptive Positive
3	3870	M	66.4	70.0	NO VARIANTS	Not affected
4	3840	F	64.3	59.2	PENDING	DNA pending

1: IonKey column (cutoff 68.4 and 55.5 respectively)

2-4: ESI low flow column (cutoff 76.2 and 75.7 respectively)

Summary

- These studies highlight the use of a novel IVD assay demonstrating the feasibility of LC-MS/MS proteomics for NBS of Wilson disease
- The false positive rate is extremely low
- FDA study shows the performance and precision of the manufactured kit were reliable, highly sensitive, and specific for targeted peptides as surrogate markers for ATP7B protein (please see separate PowerPoint slide)
- LC-MS/MS has been adopted globally in clinical laboratories and newborn screening. We anticipate the assay kit can be successfully utilized for clinical/public practice
- Other global feasibility studies are on going:
    - Sardinia (Dr. Georgios Loudianos): 41/44 cases below cutoff (manuscript in preparation)
    - Grand Canary Island, Spain (Drs. Luis García Villarreal/Antonio Tugores)
    - Costa Rica (Dr. Monica Portman Penon)

Acknowledgements

- **Key Proteo, Inc**

  Science Team:

  Claire Klippel, BS
  Jiwoon Park, PhD
  Moumita Dutta, PhD

  Business/Admin Team:

  Sean Sandin, COO
  Mark Willig, CEO

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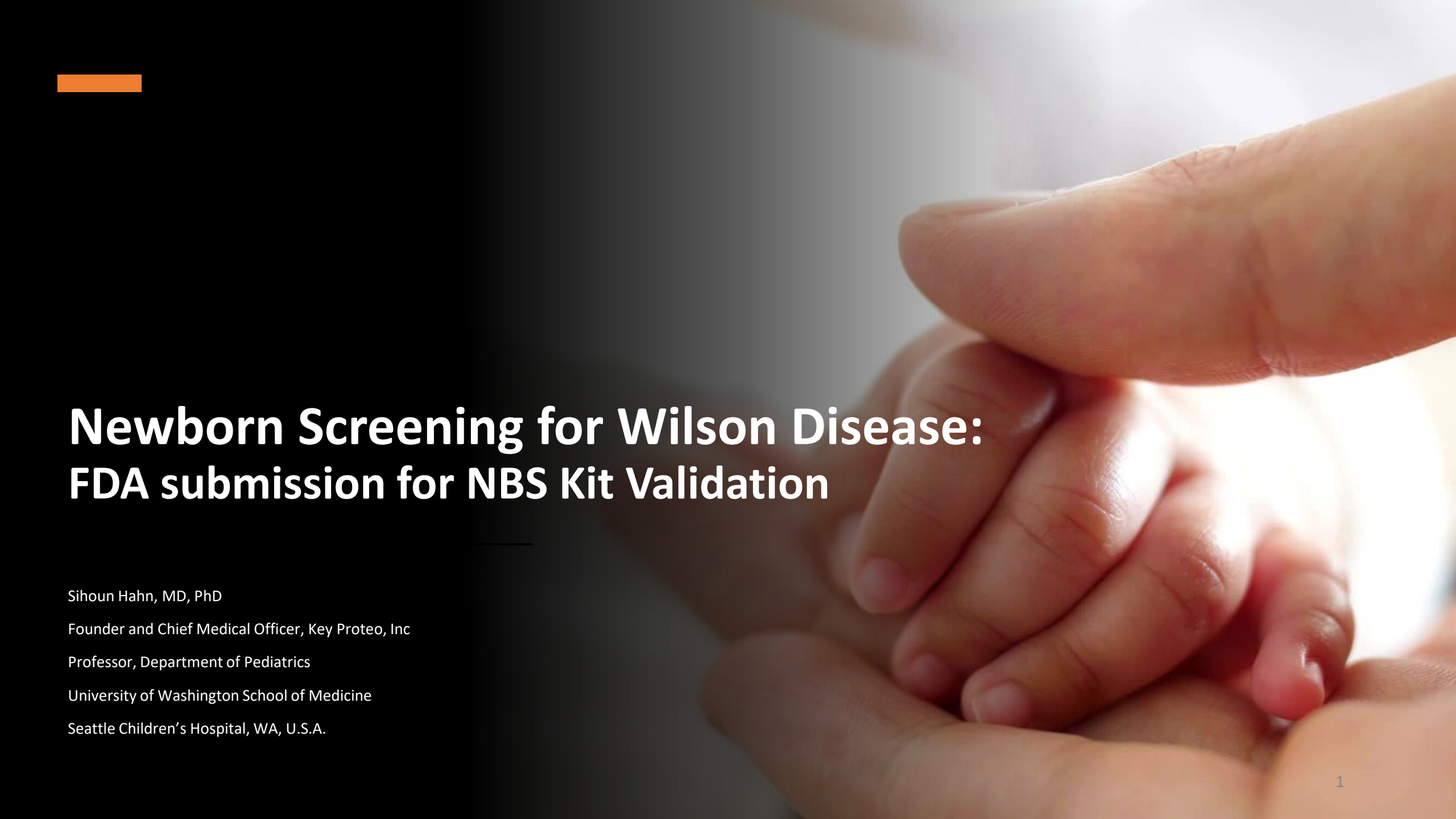
- **Alberta Precision Laboratories**

  Dennis Orton, PhD
  Tara Winstone, PhD
  Xue Chen, PhD

- **Seattle Children's Hospital**

  Hans Ochs, MD

Newborn Screening for Wilson Disease:
FDA submission for NBS Kit Validation



Sihoun Hahn, MD, PhD

Founder and Chief Medical Officer, Key Proteo, Inc

Professor, Department of Pediatrics

University of Washington School of Medicine

Seattle Children's Hospital, WA, U.S.A.

FINANCIAL DISCLOSURES

Sihoun Hahn, MD, PhD, is a member of the Seattle Children's Hospital workforce and is serving as Chief Medical Officer of Key Proteo, Inc. He is an inventor of intellectual property that has been licensed to Key Proteo, Inc. Dr. Hahn is the founder of Key Proteo, Inc. and has ownership equity interests in the company.

Key Proteo Newborn Screening Kit for Wilson Disease

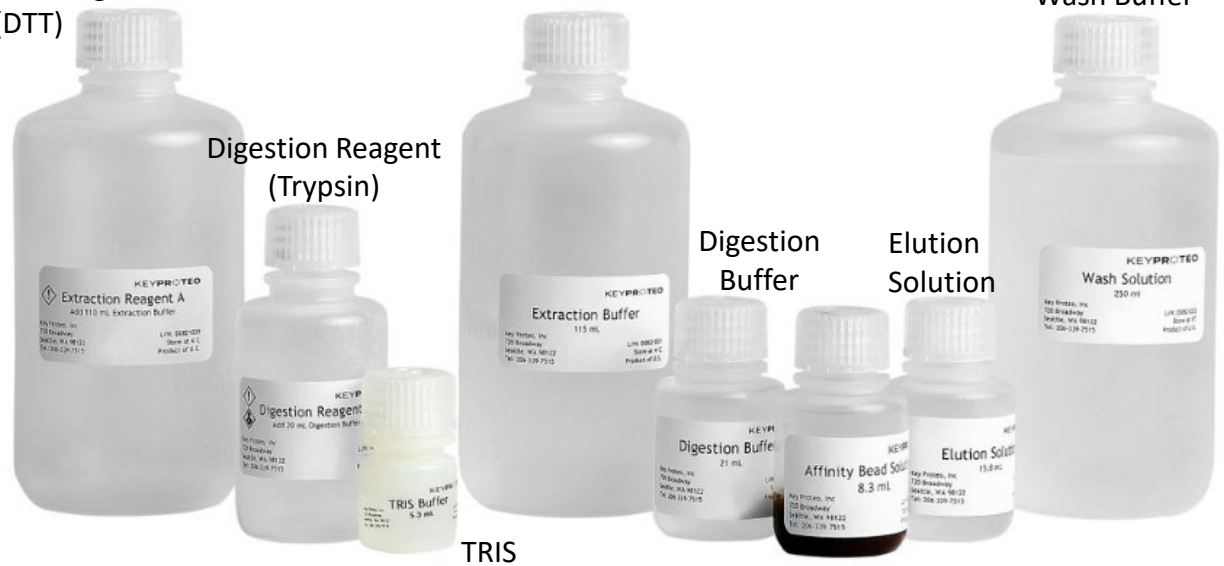
Intended Use:

- The Key Proteo Newborn Screening test is a quantitative LC-MS/MS assay, for **combined and simultaneous detection** of biomarker peptides representing (1) ATP7B, (2) BTK, (3) WASP, and (4) ADA proteins from dried blood spots. The deficiency of these proteins are diagnostic for *Wilson disease*, *X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, and ADA deficiency*, respectively.

- Key Proteo Newborn Screening assay is indicated for use in a population screening of newborns in public health labs as an initial screen for the listed conditions. Those indicated as potential patients would be guided to follow up with specialists in the indicated diseases. This will allow for additional follow up testing as deemed appropriate by healthcare providers to provide or rule out a definitive diagnosis and initiate appropriate pre-emptive treatment.

Public Health Transition: Prototype 4-plex Screening Reagents

- Prototype reagents for assay performance have been manufactured at GMO facility
- Affinity beads mixed into a single solution
- Internal Standard Peptides lyophilized into 96-well plates
- DBS Control Cards
  - 3 levels of DBS spots
  - Negative, Low, High



Internal Standard Plates



Control Cards



Prototype reagents are not FDA approved and currently intended for investigational use only

Analytical Validation Study for FDA

- *Kit was classified as De novo, Class II by FDA: First-of-its kind proteomic-based NBS IVD kit*

Reproducibility/Precision:

The precision studies were performed following the recommendations in the CLSI EP05-A3 guideline at three different laboratories. Repeatability (within-run precision), between-day precision, and reproducibility (between operators) were conducted utilizing a six level multi-analyte reproducibility panel (0,20,40,60,80, and100% human blood).

Study sites 1 and 2 were conducted over 5 days, with 2 plates per day and 3 replicate measurements of DBS samples with identical peptide concentrations by two operators for a total of 360 measurements. Each study site used 1 lot of reagents and 1 instrument.

Study site 3 was conducted over 20 days, with 2 plates per day and 3 replicates per plate by two operators for a total of 720 measurements.

Site to Site Reproducibility Study

- The repeatability and within-laboratory precision of the assay for each multiplexed peptide were determined by replicate measurement of DBS samples with 6 different peptide concentrations created by serial dilution of NHC with fish blood.
- Assessment of within-laboratory precision was done using studies based on the CLSI EP5-A3 document and the modified 20 x 2 x 2 study for internal site and 5 x 2 x 2 study for two external sites.
- The study was conducted using a total of 720 measurements for each multiplexed peptide at the internal site and 180 measurements at each external site. Each kit on the three sites was from three different lots. A two-way nested ANOVA model was used to calculate the site-specific precision estimates.
- Overall, the repeatability, between-day and reproducibility on 100% human sample showed CVs of less than 30% for ATP7B peptides in all three sites.

Peptide	Panel	Mean (pmol/L)	Intra-day (%)	Between Day (%)	Between operator (%)
ATP7B 1056	Panel A	NA	NA	NA	NA
	Panel B	56.0	6.7	6.3	6.2
	Panel C	99.7	11.3	12.1	12.0
	Panel D	168.9	9.9	13.8	13.4
	Panel E	230.9	9.7	12.1	11.8
	Panel F	312.2	12.3	13.6	12.9
ATP7B 887	Panel A	NA	NA	NA	NA
	Panel B	56.8	9.7	11.2	10.6
	Panel C	101.0	13.9	13.8	14.1
	Panel D	154.6	8.3	10.9	10.6
	Panel E	200.5	8.8	11.1	10.6
	Panel F	246.7	11.1	11.0	10.9

Analytical Validation Study for FDA

Analytical Sensitivity:

The limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ) for the test kit were determined following the recommendations in the CLSI EP17-A2 guideline with consultation of CLSI document C64 “Quantitative Measurement of Proteins and Peptides by Mass Spectrometry”, involving replicate analysis of a set of blank and low-level samples using multiple lots and a single instrument

Detection Capability (pmol/L)	N	ATP7B 887	ATP7B 1056
Limit of Blank	120	15.5	13.7
Limit of Detection	120	24.5	22.7
Limit of Quantification	180	40.2	49.6

Analytical Validation Study for FDA

Carry-Over:

Carryover was determined by replicate measurements of blank samples with and without carryover for each multiplexed peptide. The blank samples were run followed by a DBS sample with high analyte concentrations for each multiplexed peptide and repeated to evaluate change in concentration due to carryover. The carryover effects of all peptides except ADA 93 were negligible. Based on the results, a specimen with ADA concentration within or slightly higher than the borderline cutoff should be retested if a specimen with ADA concentration within the normal range is present in the same column

Target Peptide	Carryover (%)
ATP7B 887	0.2-4.1
ATP7B 1056	0.9-4.2

Analytical Validation Study for FDA

Interference:

Analytical specificity was determined in accordance with the CLSI EP7-A2 guideline. The interferents tested for the kit are as follows: Hematocrit, Unconjugated Bilirubin, Conjugated Bilirubin, Galactose, Glucose, EDTA, Heparin, Total Protein, Hemoglobin, and Triglyceride. Blood pools were made in both 100% fish blood and a 30% mixture of NHC and fish blood. Test pools were spiked with interfering substances. None of the 11 interferents tested in this experiment had a significant effect on the concentrations of all six peptides at the recommended initial interferent concentration levels (below 20%)

Interference	Recommended Concentration	Unit
Unconjugated Bilirubin	0.4	mg/mL
Conjugated Bilirubin	0.4	mg/mL
Galactose	0.6	mg/mL
Glucose	10	mg/mL
EDTA	3.4	µM
Heparin	3.3	µg/mL
Total Protein	50	mg/mL
Hemoglobin	10	mg/mL
Cholesterol	400	mg/dL
Triglycerides	1500	mg/dL
Gamma-globulin	2.5	g/dL

Analytical Validation Study for FDA

Stability:

Stressed stability test was conducted in collaboration with Nelson Laboratories (Salt Lake City, UT). The study was conducted to determine the stability of screening kit using a classical and real-time approach as described in the CLSI EP17-A2 guideline. On-board stability testing was conducted by replicate panel of eluted samples placed into the instrument sample manager and measuring across time (0, 4, 8, 24, 32 and 48 hours). The kit was stable up to 6 month or even longer at -20'C. DBS stability was determined up to 5 days at three different temperatures. ATP7B peptides were stable at all conditions with no statistically significant differences.

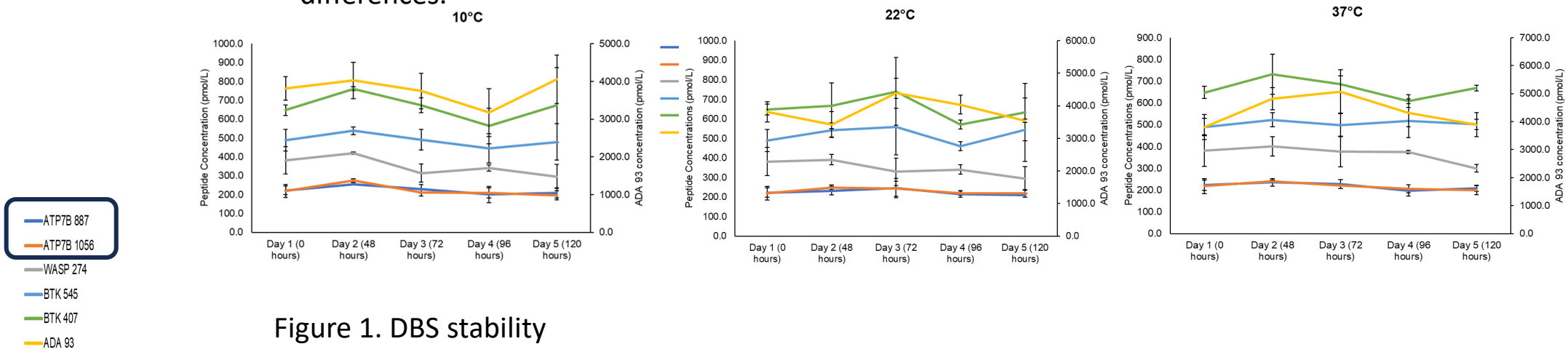


Figure 1. DBS stability

Clinical Validation Study for FDA

- A total of 3,294 newborns and 32 WD patient samples (including retrieved original NBS samples) were blindly tested at three sites (SCH, APL, KP).
  - No presumptive positive cases were detected in presumably normal newborn samples
  - All confirmed 32 positive Wilson disease cases were screened positive, and repeats were concordant with initial results
- To estimate the potential for false negative results of the test, the samples presumed to be normal (total 33 cases above the cutoff out of 3,294 newborns) were sequenced for *ATP7B* gene in a clinical molecular laboratory
  - 10 Carriers: Six (6) VUS and four (4) pathogenic variants. No second variants.
  - One uncertain case: Two variants of VUS were detected in which the peptide result was normal. The clinical significance is yet uncertain.
  - 22 cases with no variants

Additional FLEX studies

- **Upon requests, following FLEX study results can be provided:**
  - DBS punch test
  - Freeze-Thaw cycle test
  - Kit inversion test
  - Temperature/Time/Volume test
  - Hematocrit test
  - Inter-injection variation test

Summary

- FDA study demonstrated that the performance and precision of the manufactured kit was reliable, highly sensitive, and specific for targeted peptides as surrogate markers for ATP7B protein

- These studies highlighted the use of a novel IVD assay demonstrating the feasibility of LC-MS/MS proteomics for NBS of Wilson disease

- LC-MS/MS has been adopted globally in clinical laboratories and newborn screening. We anticipate the assay kit can be successfully utilized for clinical/public practice

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Key Proteo Newborn Screening Kit 1 Product Insert

Newborn Screening Kit 1

Wilson Disease | X-Linked Agammaglobulinemia | Wiskott-Aldrich Syndrome | ADA Deficiency

Reagents and Consumables for 440 specimens

Instructions For Use

Important Notice
Please read these Instructions For Use fully. If you have any questions or comments, please contact Key Proteo Customer Support at support@keyproteo.com or call (+1) 206-339-7515.

IVD
For *In Vitro Diagnostic Use*

Key Proteo, Inc.
720 Broadway
Seattle, WA 98122, USA
www.keyproteo.com

SYMBOLS GUIDE

The following symbols may be found on Newborn Screening product packaging and labeling:

	Manufacturer		*In vitro* diagnostics
	Date of Manufacture		Consult Instructions For Use
	Expiration Date		Customer Support
	Reference Item Number		Temperature Limits
	Lot Number		Do Not Reuse
	Serial Number		Control
	Caution		Keep dry
	Biohazard		Number of Tests
	Keep Away From Sunlight		

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1. Intended Use

The Key Proteo Newborn Screening Kit 1 – WD|XLA|WAS|ADA is intended for quantitative measurement of levels of specific peptides that identify high-risk populations for WD, XLA, WAS, and ADA deficiency, respectively, from newborn dried blood spot (DBS) specimens, using the XEVO-TQXS mass spectrometer as an aid in screening newborns for WD, XLA, WAS, and ADA deficiency. Reduced levels of the measured peptides may be indicative of Wilson's Disease and the inborn errors of immunity (IEI) such as XLA, WAS or ADAD. The peptides measured by the Key Proteo NBS Screening Kit – WA|XLA|WAS|ADA and their associated genetic disorders and IEI are listed below.

Protein (Abbreviation)	Disease (Abbreviation)
Copper-transporting ATPase2 (ATP7B)	Wilson Disease (WD)
Bruton's Tyrosine Kinase (BTK)	X-linked Agammaglobulinemia (XLA)
Wiskott-Aldrich Syndrome Protein (WASP)	Wiskott-Aldrich Syndrome (WAS)
Adenosine Deaminase (ADA)	Adenosine Deaminase Deficiency (ADAD)

2. Indications for Use

The Key Proteo Newborn Screening Kit 1is a quantitative LC-MS/MS assay, for combined detection of ATP7B, BTK, WASP, and ADA proteins from dried blood spots. The deficiency of each of these proteins indicates high risk for Wilson's Disease, X-linked Agammaglobulinemia, Wiskott-Aldrich Syndrome, and ADA deficiency, respectively. The Key Proteo Newborn Screening assay is indicated for use in a population screening of newborns as an initial screen for the listed conditions. Those indicated as potential patients would be guided to follow up with specialists in the indicated diseases. This will allow for additional follow up testing as deemed appropriate by healthcare providers to provide or rule out a definitive diagnosis and initiate pre-emptive appropriate treatment.

Warnings: For *In Vitro Diagnostic Use*

3. Summary and Explanation of the Test

Wilson Disease (WD) is an autosomal recessive copper transport disorder with an estimated incidence of 1 in 30,000 individuals and with a carrier frequency of 1 in 90 [1]. WD is caused by mutations in the *ATP7B* gene, which encodes a copper-transporting ATPase. Copper accumulation in WD is present at birth and continues until toxic levels in the brain and liver produce noticeable symptoms which eventually lead to disease diagnosis. Symptoms usually present in the first decade of life, with most cases occurring between the ages of 5 and 35 years old [2]. Hepatic symptoms predominate in younger patients in their first decade of life, and neurological symptoms occur in older patients in the second or third decade of life [3]. Patients left undiagnosed and untreated during this accumulation phase develop irreversible brain damage, often in the form of Parkinsonism, and require liver transplants due to liver cirrhosis. Early treatment, with either a chelating agent such as Trientine or with zinc salts, has been proven to be highly effective in preventing these negative sequelae and allows patients to live essentially normal lives [4]. Nevertheless, since WD is rare, there is a significant challenge in rapid screening, thus treatment is often started only after the development of severe and permanent complications. Previous pilot studies using ceruloplasmin as a potential biomarker were unsuccessful due to high false positive and negative rates [5].

There are >1300 variants in *ATP7B* reported worldwide [6, 7] (www.varsome.com). Most mutations are rare, and the five most prevalent mutant alleles are estimated to be responsible for ~70% of the disease spectrum [8]. The two most common mutations, p.H1069Q (~35% in the European population) and p.R778L (~30% in the East Asian population) [8-10], result in markedly decreased levels of ATP7B protein, presumably due to enhanced degradation [10-12]. This is in line with observations that disease-causing missense mutations [13-16], protein-truncating nonsense mutations (~13% of known point mutations) [17], and frameshift mutations [8] result in the absence or decay of mRNA [18, 19] and therefore absent or diminished levels of the protein. ***Taken together, it is expected that most patients with WD have an absence or decreased level of ATP7B protein***. Our previous studies have confirmed this protein reduction in patient samples studied thus far [20-23].

Inborn Errors of Immunity (IEI) are a group of over 416 genetic disorders that compromise the health of affected individuals due to an improperly functioning or wholly absent immune system. These conditions often arise from genetic variants that lead to deficiencies in the abundance and/or activity of various proteins critical for immune function in the crucial time between birth and diagnosis. During this time period, serious complications related to IEI can emerge, including profound and frequent infections, autoimmunity, malignancy, and death. Although individually quite rare with varying frequency, the combined prevalence of all IEI is estimated to be about 1 in 1,200 [24-26]. Fortunately, if diagnosed early and accurately, appropriate treatments can dramatically improve, and in some cases, save the lives of the patients [27, 28]. Depending on the disorder, effectively curative methods exist such as hematopoietic stem cell transplantation (HSCT) and gene therapy [28-34]. Undoubtedly, early detection of IEI is essential to manage and prevent potentially life-threatening infections and chronic negative sequelae [35, 36].

(a) X-Linked Agammaglobulinemia (XLA) is *the most common primary immunodeficiency in men*. It is caused by a single genetic defect encoding **Bruton's tyrosine kinase (BTK) protein** and occurs with an incidence of 1:200,000 [37]. Loss of function of this kinase in males leads to a block in B cell development and B cell lymphopenia. Patients are therefore unable to make productive antibody responses and are prone to severe, life-threatening infections. Most patients with XLA who receive immunoglobulin on a regular basis will be able to lead relatively normal lives [27]. They do not need to be isolated or limited in

their activities. For **XLA**, ~60% of patients lack expression of BTK, and another 20-25% demonstrate markedly decreased levels [38, 39]. We have confirmed this reduction in 97% of patients from previous studies [21, 22].

(b) Wiskott-Aldrich Syndrome (WAS) is an X-linked congenital immunodeficiency in which affected males have very small, dysfunctional platelets and consequently have moderate to severe thrombocytopenia and a tendency to have bleeding problems with a combined immunodeficiency caused by defects in **WAS Protein (WASP)** expression [40]. It has an estimated incidence between 1-10 in 1 million [41]. The only permanent cure for WAS is transplantation of stem cells from bone marrow, peripheral blood, or cord blood. Thirty years ago, WAS was a fatal disorder with a life expectancy of only two to three years. Follow-up of the earliest WAS bone marrow transplant recipients for more than 30 years has demonstrated that this therapy is curative [31, 40]. The recent success of gene therapy for WAS holds promise for being the treatment of choice for this disease in the future [42]. In **WAS** patients, ~60% have a complete absence of WASP protein expression and another ~35% demonstrate decreased levels [43, 44]. We have confirmed this reduction in 100% of patients from previous studies [21, 22].

(c) Adenosine Deaminase (ADA) is expressed in erythrocytes, lymphoid cells, and plasma, and is mutated in ADA-deficient $T^-B^-NK^-$ SCID [45]. Inherited ADA deficiency causes a severe form of SCID that may have a somewhat variable phenotypic spectrum, with 10%-15% of patients having a 'delayed' clinical onset presenting by 6 to 24 months, and a smaller percentage of patients having a 'later' onset, diagnosed from age 4 years to adulthood. This form of SCID can be missed by the current SCID NBS method [46]. We have confirmed this reduction in previous studies [21].

4. Principles of Procedure

In the reagent Kit, protein concentrations are analyzed by measuring the amounts of representative peptides that are specific to the targeted protein. An internal standard peptide nearly identical to the target sequence is included at a known concentration and analyzed along with the peptide biomarker. The peptide concentration in blood is then calculated using the ratio of the peptide in the dried blood spots (DBS) to the internal standard.

From each DBS sample, proteins in the specimen are extracted using 50 mM ammonium bicarbonate solution. Disulfide bond reduction is performed using 2 M DTT. Trypsin enzyme is added to initiate digestion of proteins to peptides. Heavy isotope-labeled synthetic peptide internal standards (IS) are incorporated at optimized concentrations for quantification by MS/MS. Target peptides are then captured and enriched using anti-peptide monoclonal antibodies immobilized on magnetic protein G beads. Samples are incubated overnight at 4˚C with shaking. After incubation, anti-peptide MAb beads containing captured peptides are collected using a magnet and washed to remove non-target peptides. Finally, peptides are eluted with 30 µL of 5% acetic acid/3% acetonitrile. Since the internal standard and target peptide are chemically identical, any loss of target peptide (incomplete MAb capture, absorption to vessel walls, etc.) is accounted for. Target peptide-containing supernatant is transferred to a new well for subsequent MS/MS analysis.

LC-MS/MS is performed using a Waters Xevo TQ-XS. LC runs are performed with an elution gradient using H_2O + 0.1 % formic acid and acetonitrile + 0.1% formic acid before column washing and re-equilibration for a total run-time of 2.4 minutes for multiplexed analysis of 12 peptides (six endogenous peptides extracted from DBS and six isotopically labeled IS) covering 4 target conditions. The mass spectrometer is operated in MS/MS mode that allows the first mass-selective filter (quadrupole) to pass the target peptide-derived parent ion. This is followed by collision-induced dissociation of the peptide in the second quadrupole followed by a third quadrupole to allow a specific peptide-derived fragment to pass to the detector (triple-quadrupole MS/MS). This is referred to as single reaction monitoring mode (SRM). A collection of SRMs, one for each target peptide, is cycled rapidly so that all target peptides are detected in the same LC-MS/MS run (multiple reaction monitoring, MRM). The Reagent Kit contains the necessary reagents and consumables for completion of the assay before LC-MS/MS analysis.

5. Kit Contents

Each reagent kit contains sufficient reagents for 440 newborn specimens and quality controls, totaling 1760 tests as each specimen is tested for up to 4 different protein deficiencies. The expiration date for the unopened kit is on the outer label. An expiration date is noted on the labels of the individual kit components. The components are provided in two boxes- one cold (2-8 °C) box containing reagents and one room temperature (15-30 °C) box containing plastics and consumables. The kit components along with their quantity and storage conditions are listed in **Table 1** and **2.**

5.1. Materials Provided:

Table 1. Refrigerated (2-8 °C)/Frozen box contents.

Item	Composition	Delivery State	Number in Kit	Initial Storage	Storage After Initial Use
DBS Calibrator Cards	QC Card: Negative	Dried Blood Spot	1 Card x 5 spots	-20°C	-20°C
	QC Card: Low	Dried Blood Spot	1 Card x 5 spots	-20°C	-20°C
	QC Card: High	Dried Blood Spot	1 Card x 5 spots	-20°C	-20°C
Internal Standard	IS Mix in Deep Well Plate	Lyophilized	5 plates	-20°C	-20°C
Extraction Reagent A	DTT	Solid	1 Bottle x 101.8 mg	2-8°C	Aliquot and store at -20°C
Extraction Buffer	0.1% Triton X-100 in Ammonium Bicarbonate	Liquid	1 Bottle x 115 mL	2-8°C	
Digestion Reagent A	Trypsin	Solid	1 Bottle x 20 mg	2-8°C	Aliquot and store at -20°C
Digestion Buffer	50 mM Acetic Acid	Liquid	1 Bottle x 21 mL	2-8°C	
Tris Solution	1M Tris pH 8.0	Liquid	1 Bottle x 5.3 mL	2-8°C	2-8°C
Bead Mix	In 1X PBS + 0.03% CHAPS	Liquid	1 Bottle x 9.2 mL	2-8°C	2-8°C
Wash Solution	0.1X PBS + 0.01% CHAPS	Liquid	1 Bottle x 250 mL	2-8°C	2-8°C
Elution Solution	H_2O + 5% acetic acid + 3% ACN	Liquid	1 Bottle x 15.8 mL	2-8°C	2-8°C

Table 2. Room temperature (15-30 °C) box contents.

Item	Number In Kit
50 mL Reservior	2
10 mL Reservior	3
10 mL Reservior PPE	1
DWP for Extraction	5
MS Plate	5
Plate Seal	20
MS Plate Seal	5

5.2 Internal Standard Concentrations

Internal standard peptides are utilized to indicate the concentrations of the target peptides in the blood specimen. These internal standards are mixed in the correct ratios and lyophilized into 96-well *Internal Standard Plates*. Each well of the *Internal Standard Plate* contains enough internal standard peptide for 1 experiment. The amounts of each peptide lyophilized into each well is listed below in **Table 3**.

Table 3. Internal Standard amounts in each well.

Peptide	Femtomole Peptide
BTK 407	25
WAS 274	25
ATP7B 1056	5
ATP7B 887	5
BTK 545	50
ADA 93	100

5.3 Calibration

Included in each concentration calculation is a constant factor that calibrates the measured peptide concentration values between lots. This ensures consistency of the measured concentrations between lots and is designed to help maintain consistent cutoffs. The constant value by which the blood peptide/internal standard ratio should be multiplied is provided in a lot-specific quality control certificate.

5.4 Quality Control DBS

The quality control dried blood spots include 3 levels of control material: QC negative, QC low, and QC high. The composition of the quality control DBSs is summarized in **Table 4** below. The peptide concentrations in the QC dried blood spots are provided on a lot-specific quality control certificate included in each assay kit. Each laboratory should establish its own mean and acceptable range.

Table 4. Quality Control Materials.

Quality Control Level	Composition
QC Negative	Fish Whole Blood drawn with Sodium Heparin anticoagulant before spotting
QC Low	A mixture of Normal Human and Fish Whole Blood drawn with Sodium Heparin anticoagulant before spotting
QC High	Human Whole Blood drawn with Sodium Heparin anticoagulant before spotting

5.5 Materials required but not provided:

- DBS puncher
- Personal protective equipment: gown and gloves.
- Adjustable single-channel pipettes: as needed.
- Single-channel pipette tips: as needed.
- Multichannel pipettes: 10 µL, 100 µL, 300 µL, 1200 µL
- Multichannel pipette tips: 10 µL, 100 µL, 200 µL, 1200 µL
- Alpaqua Magnum EX universal magnet plate, A000380 (Beverly, MA) or equivalent.
- KimWipes, Kimberly-Clark, Fisher Scientific. (Chicago, IL) or equivalent.
- Vortex Mixer, Four E's (Amazon, M/N: MI0101002) or equivalent.
- Orbi-Shaker MP, BenchMark Scientific (Genesee Scientific) or equivalent.
    - For use at 15-30 ℃ and 2-8 ℃
- Incu-Mixer MP Plate Shaker, BenchMark Scientific (Genesee Scientific) or equivalent.
    - For use at 37+/-1 ℃
- Centrifuge 5810, Eppendorf (AG, Germany) or equivalent.
    - Capable of centrifuging deep well plates/microtiter plates
- Waters Xevo TQ-XS MS with ESI source and Low-Flow probe connected to Waters M-Class Gradient and Loading pumps, Waters (Milford, MA).
- nanoEase M/Z Peptide Colum; P/N 186009257, Waters (Milford, MA).
- 2x nanoEase loading column; P/N: 186009251; Waters (Milford, MA).
- Acetonitrile (no. A955, LCMS optima grade); Thermo Fisher Scientific (Waltham, MA) or equivalent; Stored at 15-30 °C.
- Water (no. W6, LCMS optima grade); Thermo Fisher Scientific (Waltham, MA) or equivalent; Stored at 15-30 °C.
- Formic Acid (no. A117-50, LCMS optima Grade); Fisher Scientific (Waltham, MA) or equivalent; Stored at 2-8 °C.

6. Warnings and Precautions

- For in vitro diagnostic use.
- For professional use only.
- The Key Proteo Newborn Screening Kit 1 should be used by adequately trained personnel.
- Gloves should be worn whenever working with blood samples. All blood samples, containers, and materials that contact blood should be handled as if capable of transmitting infectious disease and discarded into a biohazard waste container after use.
- This kit contains quality control DBS that were produced using human blood specimens. All quality control DBS were tested for HIV, Hepatitis B, Hepatitis C, and other bloodborne pathogens by FDA-approved or equivalent methods and found to be negative for all infectious agents. However, all recommended precautions for handling blood specimens as specified in the document provided by U.S. Health and Human Services titled "Biosafety in Microbiological and Biomedical Laboratories" should be followed.
- *Internal standard plates, QC dried blood spots, and lot-specific QC reports are all lot specific and should not be mixed between lots.*
- Disposal of all waste should be in accordance with local regulations.
- Ensure that materials from the Key Proteo Newborn Screening Kit 1 are treated as biohazard waste.
- Do not use any reagent kit components after their expiration dates.
- Do not use any damaged reagent kit components.
- Store all reagent kit materials as described in section 5.1, Materials Provided.
- Keep work areas clean per standard practices.
- Wash and dry hands before and after testing. Wear gloves and appropriate PPE.

7. Limitations

- Test results are intended to be used in conjunction with clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, and clinical evaluation as appropriate.
- Reduced or absent protein concentrations must be confirmed by standard diagnostic methods, such as gene sequencing.
- This test is a screening test and is not intended to diagnose the genetic conditions listed herein.
- As with other screening tests, negative results do not rule out the diseases being screened.
- Conditions that are known to cause anomalous results are[47]:
    - Specimen spot not uniformly saturated with blood.
    - Specimen spot punched too close to the edge of the blood spot.
    - Poorly collected and improperly dried specimens.
    - Non-eluting blood spot due to deterioration of specimen caused by exposure to heat and humidity.
    - NBS samples collected from premature infants or sick/transfused infants may require mandated repeat specimen.
- The Key Proteo Newborn Screening Kit 1 may result in:
    - False positives by detecting carriers for the targeted diseases.
    - False negatives if disease-causing mutations do not affect protein concentration.

    - Please also refer to the Section titled "Procedural Notes".
- The test is specific for the targeted peptides. Other peptides that may be present in the blood do not react in/are not identified by the test.
- Certain regions or demographics may have differences in average protein concentrations or the incidences of false positives and negatives.
- The performance of the Key Proteo Newborn Screening Kit 1will be determined using the procedures provided in these Instructions For Use. Failure to follow these procedures and precautions as directed may alter test performance.
- Improper sample handling (through collection, storage, or transport) may impact results or lead to invalid results.
- This reagent kit cannot identify peptide levels associated with diseases other than those stated in this Instructions For Use.

8. Specimen Collection, Storage, and Handling

Blood specimens should be taken using the standard procedures utilized by public health workflows. Neonatal screening programs differ from one another in the type of specimen required. In the United States, the recommendation is that a blood spot, approximately 12.7 mm (0.5 inch) in diameter, be collected by heel prick and spotted onto filter paper. Blood from a newborn heel prick is usually collected 24-48 hours after birth. However, in some screening programs, the specimen from the neonatal heel prick may be collected 2-6 days after birth. Consult local regulations for appropriate timing and screening specimen collection. The manufacturer recommends following the procedures listed in the CLSI document number NBS01 - A6- "Blood collection on filter paper for newborn screening programs; Approved standard"- Sixth edition (2013)[47] and some important points are mentioned below:

- Ensure that the expiration date of the blood collection card has not passed.
- Wipe the newborn skin with 70% isopropanol and allow the skin to dry.
- Puncture the infant's heel with a heel incision device or a sterile lancet by making a standardized incision of 1.0 mm deep. Ensure that the puncture does not exceed 2.0 mm in depth since a deep incision may cause bone damage in small infants.
- Wipe away the first drop of blood with a sterile gauze pad and allow a large drop of blood to form. Touch the filter paper against the large drop of blood and allow a sufficient quantity of blood to soak through and fill the entire pre-printed circle. Examine both sides of the paper to ensure that the blood uniformly penetrated and saturated the filter paper.
    - o Do not excessively squeeze the puncture since it may cause hemolysis of the specimen or result in a mixture of tissue fluids with the specimen and might adversely affect the assay result.
    - o Do not apply successive blood drops to the same printed circle or already partially dried spots can result in "caking".
- Allow the blood specimen to air dry on a horizontally level, non-absorbent, open surface for at least 3 hours at an ambient temperature, away from direct sunlight. Ensure that the specimens are not stacked to avoid cross-contamination.
- Ensure that the required information on the dried blood spot card is completed which includes:
    - o Last name, First name, Sex, Birth date, Birth weight and Patient identification number
    - o First and last name of mother
    - o Date of specimen collection
    - o Name and address of the submitter
    - o Name and phone number of the physician
    - o Name of the newborn screening program and address
    - o Each card should have a unique serial number
- Follow the basic triple packaging system i.e., blood absorbed into paper, a fold-over flap or inner envelope, and an outer envelope of high-quality paper. Ensure that the local regulation and institutional policies are followed when shipping dried blood spot specimens.
- Transport the dried blood spot specimens within 24 hours of collection unless otherwise directed by the newborn screening laboratory.
- Humidity and moisture are detrimental to the quality of the dried blood spot. Special attention must be paid to the storage and transportation conditions of the dried blood spot specimens. Storage of specimens in an environment with elevated temperatures and humidity may increase the risk of false positive results.

8.1. Target Peptide Stability in Dried Blood Spots

This plan governs the determination of DBS stability for the Key Proteo Newborn Screening Kit 1 before analysis. As this is a multiplexed test, stability was determined for each of the six peptide targets. Assay design involves replicate analysis of a panel of eluted samples and measured across time up to 5 days at 10°C, 25°C, and 37°C. Through this protocol, it was determined how long the DBS samples can be expected to measure consistent concentrations over time at different temperature conditions. Quality control and 100% normal human control blood(NHC) , samples were processed per the IFU. Three (3) replicates of each sample were injected at each subsequent day and change in concentrations over time were monitored.

All six target peptides were stable for up to 5 days at all three different temperatures (10°C, 22°C and 37°C) with no statistically significant differences.

9. Assay Procedure

The Key Proteo Newborn Screening Kit 1 assay procedure is detailed below.
**NOTE*: All frozen and refrigerated reagents, components, and samples must be brought to room temperature (15-30°C) before use.*

9.1. Plate and Specimen Preparation

- Wear nitrile gloves and clean work bench with 70% Ethanol.
- Initialize automated DBS puncher or equivalent for use.
- If using a standard hole puncher, clean standard hole puncher and tweezer by spraying and wiping with 70% EtOH.
- Pre-heat Incu-Mixer MP Plate Shaker or equivalent to 37+/-1°C.
- Punch out controls and samples into wells (1/8-inch punches from filter paper disks).
    - To minimize variation in controls it is advisable to avoid punching solely from the perimeter of the dried blood spot.
    - A recommended punching layout is represented below where the three punches needed for each well can be taken from each of three DBS sections below (**Figure 1**).

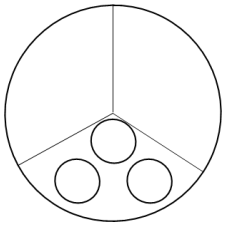


Figure 1. Diagram of recommended DBS punching locations. Three punches can be taken from each of three DBS sections.

Table 5. Recommended Quality Control DBS punch location

A12	IS
B12	IS
C12	QC Negative
D12	QC Low
E12	QC High
F12	QC Negative
G12	QC Low
H12	QC High

- A sample plate layout is shown below in **Figure 2**.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	IS
B	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	IS
C	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC Neg
D	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC Low
E	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC High
F	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC Neg
G	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC Low
H	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	NBS	QC High

Figure 2: Recommended plate layout

- Thaw Extraction Solution aliquots or make Extraction Solution per DBS Protein Extraction protocol below.
- For each DBS sample, punch 3 x 3.2-mm punches (equivalent to ~10.5 µl blood per punch). DBS spots are punched (using Panthera or equivalent) or placed (using tweezers) into 96-well Masterblock Extraction Plate wells.
- Generate Plate Layout document (each sample has a designated well location, i.e. A1, A2…).

8.2. DBS Protein Extraction

- Add 110 mL of Extraction Buffer into bottle containing Extraction Reagent A.
- Invert 10 times to dissolve and mix.
- Transfer resulting Extraction Solution to 50 mL Reagent Reservoir as needed.
- Add 200 µL of this Extraction Solution into each well.
- Seal plate with a new foil plate seal.
- Place the plate into Incu-Mixer MP Plate Shaker or equivalent and incubate at 37+/-1°C for 25-35 min at 1000 RPM.
- NOTE: Store remaining Extraction Solution in 21-ml aliquots in -20°C freezer.
    - To thaw:
        - remove aliquot from -20°C freezer.
        - Thaw to 15-30°C using one of the following options: let sit at 15-30°C, or incubate at 37+/-1°C.

8.3.Trypsin Digestion

- Transfer 20 mL of Digestion Buffer into bottle containing Digestion Reagent A. ***Do Not Vortex!***
- Pipette to dissolve and mix.
- Transfer resulting Digestion Solution to 10 mL Reagent Reservoir.
- Remove plastic plate seal, then add 37.5 µL of Digestion Solution to each well.
- Seal plate with new foil plate seal.
- Incubate mixture on Incu-Mixer MP Plate Shaker or equivalent at 37+/-1°C for 2 hours +/- 10 min to digest.
    - NOTE: Store remaining Digestion Solution in 4-ml aliquots in -20°C freezer.
    - To thaw:
        - remove aliquot from -20°C freezer.
        - Thaw by letting sit at 15-30°C.

8.4. Peptide Enrichment

- Remove plate seal from the Internal Standard Plate.
- Remove foil plate seal from the Extraction Plate, then transfer 200 µL of the resulting DBS digest to its corresponding well on the Internal Standard Plate.
- Remove TRIS Buffer from 2-8°C refrigerator and mix gently by swirling or pipetting up/down.
    - Do Not Vortex.
- Transfer TRIS Buffer into 10 mL reservoir for ease of addition to plate.
- To each sample, add 10 µL of TRIS Buffer.
- Mix for 1-3 minutes on Orbi-Shaker MP at 1000 RPM at 15-30°C.

- Remove Affinity Bead Solution from 2-8°C refrigerator and mix gently by pipetting.
- Transfer Affinity Bead Solution into 10 mL Affinity Bead Reservoir for ease of addition to plate.
- To each sample, add 18 µL of Affinity Bead Solution.

- Seal plate with a new foil plate seal (foil if leftover lyo spots).
- Incubate samples overnight on Orbi-Shaker MP at 1000 RPM at 2-8°C to allow for peptide capture.

8.5. Washing Beads

- The next day, remove foil plate seal from Internal Standard Plate, then place plate on Alpaqua Magnum EX magnetic plate rack and leave for 1-3 min to collect beads.
- Remove Wash Solution from 2-8°C refrigerator and invert 10-15 times to mix.
- Transfer Wash Solution to 50 mL reagent reservoir to aid in transfer to plate.
- Transfer supernatant from beads to corresponding wells in new DWP. Store plate containing transferred digest in -20°C freezer.
- Remove plate from Alpaqua Magnum EX magnetic plate rack.
- Add 220 µL of Wash Solution to each well.
- Pipette up/down 5-10 times to mix.
- Place plate on Alpaqua Magnum EX magnetic plate rack and leave for 1-3 min to collect beads.
- Remove supernatant from beads and discard.
- Remove plate from Alpaqua Magnum EX magnetic plate rack.
- Repeat wash by adding 220 µL of Wash Solution to each well.
- Pipette up/down 5-10 times to mix.
- Place plate on Alpaqua Magnum EX magnetic plate rack and leave for 1-3 min to collect beads.
- Remove supernatant from beads and discard.
- Remove plate from Alpaqua Magnum EX magnetic plate rack.

8.6. Peptide Elution

- Add Elution Solution to 10ml reservoir.
- After discarding the final supernatant, add 30 µL of Elution Solution to each well.
  - NOTE: Pipet up/down 10-20 times (until fully homogenized).
- Seal plate with a new foil plate seal.
- Shake for 4-6 minutes on Orbi-Shaker MP or equivalent at 1000 RPM at 15-30°C to elute peptides.
- Centrifuge in Eppendorf 5810 or equivalent at 600 RPM for 15-25 seconds.
- Remove plate seal, then place plate on Alpaqua Magnum EX magnetic plate rack. Leave for 1-3 min to collect beads.
- Transfer 14 µl of eluted peptide solution into corresponding 96-well MS Plate.
  - Take care not to disturb or pipette the beads collected on the bottom of the well.
- NOTE: If beads are disturbed or aspirated, return beads to well, wait 20-30 seconds to allow to settle, ensure pipet is placed in center of well, then re-attempt aspiration.
- Seal plate with new MW Waters plate seal.
- Centrifuge in Eppendorf 5430 centrifuge or equivalent at 3000 rpm for 25-35 seconds.
- Transfer plate to MS for analysis.

8.7. Liquid Chromatography Conditions and Peptide Transition List

[Liquid Chromatography (LC) Inlet Method]

- The provided Key Proteo Newborn Screening Kit 1 standard LC conditions are below.
- Run time, load time, and LC mode are listed in **Table 6**.

Table 6. Inlet method run time, loading time, and mode.

Run Time (Min)	2.4
Loading Time (Min)	1
Mode	Trapping

- Trapping Conditions for both the gradient and loading pumps are listed in **Table 7**.

Table 7. LC Inlet method trapping conditions.

Trapping - Gradient Pump			
Time (min)	Flow (µL/min)	% A	% B
Initial	8	95	5

Trapping - Loading Pump			
Time (min)	Flow (µL/min)	% A	% B
Initial	40	98	2

- Analytical conditions for both the gradient and loading pumps are listed in **Table 8**.

Table 8. LC Inlet method gradient conditions

Analytical - Gradient Pump			
Time (min)	Flow (µL/min)	% A	% B
Initial	8	95	5
0.1	8	95	5
0.3	8	80	20
0.55	8	80	20
0.95	8	70	30
1.3	8	5	95
1.75	8	5	95
1.9	8	95	5
2.4	8	95	5

Analytical - Loading Pump			
Time (min)	Flow (µL/min)	% A	% B
Initial	40	98	2

- Trap valve manager settings should be set to “Toggle” as shown in **Figure 3**.

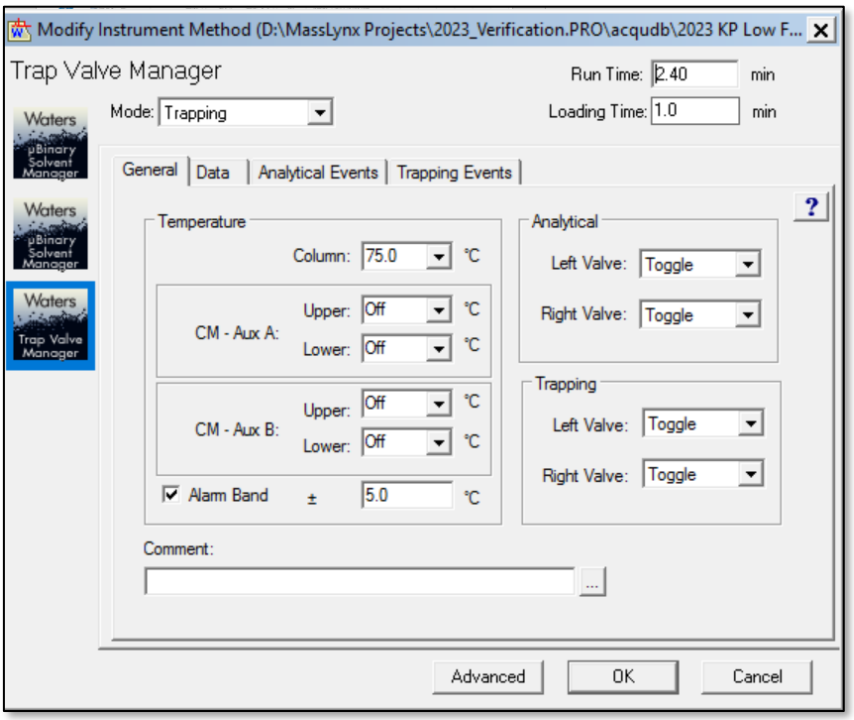


Figure 3: Trap Valve Manager Settings

- µSample Manager settings should be set as in **Figure 4**.

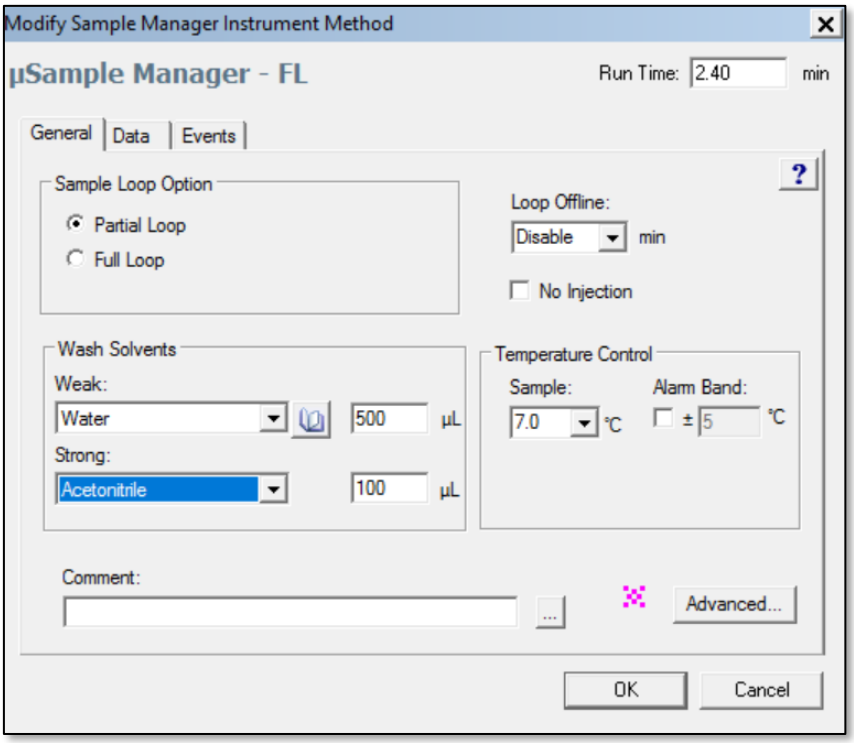


Figure 4: µSample Manager Settings

- Trapping mode advanced settings should be selected as shown in **Figure 5**.

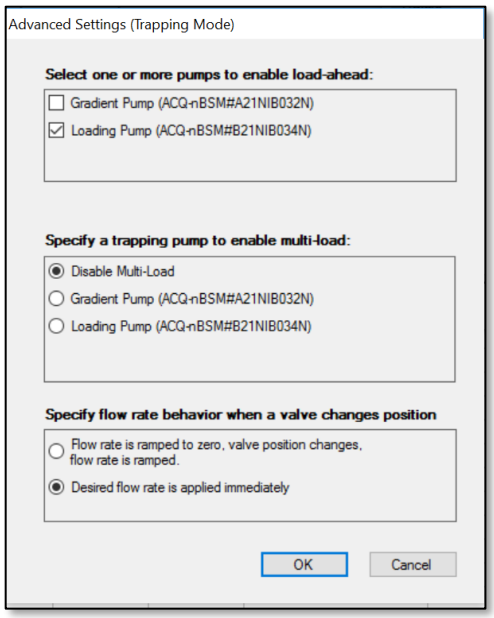


Figure 5. Advanced Trapping Mode Settings

[Mass Spectrometry (MS) Method]

- The provided Key Proteo Newborn Screening Kit 1 standard MS method conditions are below.
- **Table 9** lists the parent ions, fragment ions, cone voltages, and collision energies for each target peptide.

Table 9. Transition information for the Key Proteo Newborn Screening Kit 1 Standard MS Method.

		Parent Mass (m/z)	Fragment Mass (m/z)	Cone Voltage (kV)	Collision Energy (kV)	Fragment
Endogenous (Light)	ATP7B 887 L	523.6	558.2	0	14	y5+
		523.6	671.4	6	14	y6+
		523.6	695.3	0	14	b7+
		523.6	772.6	2	12	y7+
Internal Standard (Heavy)	ATP7B 887 IS	526.3	566.2	0	14	y5+
		526.3	679.4	6	14	y6+
		526.3	695.3	0	14	b7+
		526.3	780.6	2	12	y7+
Endogenous (Light)	ATP7B 1056 L	712.6	827.4	0	18	y17++
		712.6	877.0	0	18	y18++
		712.6	926.5	0	18	y19++
Internal Standard (Heavy)	ATP7B 1056 IS	715.2	831.4	0	18	y17++
		715.2	881.0	0	18	y18++
		715.2	930.5	0	18	y19++
Endogenous (Light)	WASP 274 L	761.0	650.7	8	23	y6
		761.0	751.5	0	22	y7
		761.0	864.6	8	24	y8
		761.0	1063.5	0	24	y10+
Internal Standard (Heavy)	WASP 274 IS	764.9	658.7	8	23	y6
		764.9	759.4	0	22	y7
		764.9	872.4	8	24	y8
		764.9	1071.5	0	24	y10+
Endogenous (Light)	ADA 93- L	525.4	403.6	5	12	y3+
		525.4	502.3	5	14	y4
		525.4	548.3	10	14	b5
Internal Standard (Heavy)	ADA 93 IS	530.5	413.6	5	12	y3+
		530.5	512.3	5	14	y4
		530.5	548.3	10	14	b5
Endogenous (Light)	BTK 545 L	781.8	828.4	0	28	y8+
		781.8	1187.5	0	20	y11+
		781.8	1300.6	0	28	y12+
Internal Standard (Heavy)	BTK 545 IS	785.8	836.4	0	28	y8+
		785.8	1195.5	0	20	y11+
		785.8	1308.6	0	28	y12+
Endogenous (Light)	BTK 407 L	568.1	402.3	2	16	y4+
		568.1	446.8	8	13	y9++
		568.1	892.5	2	16	y9+
Internal Standard (Heavy)	BTK 407 IS	571.9	410.3	2	16	y4+
		571.9	450.8	8	13	y9++
		571.9	900.5	2	16	y9+

[MS Analysis Procedure]

1. Open the MassLynx environment.

2. Open or generate the necessary sample list.
    a. Navigate to File -> Open -> *Select the relevant sample list.*
    b. Alternatively, generate the necessary sample list by entering the information for all analyte and QC samples.

3. Ensure the correct columns are present in the MassLynx Window.
    a. File Name
    b. Bottle/Vial
    c. Injection Volume
    d. Inlet Method
    e. MS File
    f. MS Tune File
    g. Sample Type
    h. ATP7B 887 (CONC A)
        i. If this column is headed with "CONC A", right click the column and use the "Properties" selection to rename the column to the peptide name.
    i. ATP7B 1056 (CONC B)
        i. If this column is headed with "CONC B", right click the column and use the "Properties" selection to rename the column to the peptide name.
    j. WASP 274 (CONC C)
        i. If this column is headed with "CONC C", right click the column and use the "Properties" selection to rename the column to the peptide name.
    k. ADA 93 (CONC D)
        i. If this column is headed with "CONC D", right click the column and use the "Properties" selection to rename the column to the peptide name.
    l. BTK 545 (CONC E)
        i. If this column is headed with "CONC E", right click the column and use the "Properties" selection to rename the column to the peptide name.
    m. BTK 407 (CONC F)
        i. If this column is headed with "CONC F", right click the column and use the "Properties" selection to rename the column to the peptide name.
    n. Quan Meth
    o. File Text
    p. If these fields are not present Right Click on the column heading bar and select "Customize Display". Then select the fields above.

4. Enter Standard Methods for control of MS, LC, and Tune page (**Figure 6**).
    a. Inlet File -> *Standard LC Method*
    b. MS File -> *Standard MS Method*
    c. MS Tune File -> *Standard Tune File*
        i. This file should be generated by Waters upon installation or calibration.

5. Enter Sample Specific Information.
    a. Under Sample Type
        i. Designate QC samples as "QC" using the dropdown menu.
        ii. Designate all other samples as "Analyte"
            1. Use Right Click -> Fill down to rapidly fill menus.

6. Enter Lot Specific QC Information.

a. Concentration values for each peptide will be provided on a lot-specific QC certificate.
b. Values will be provided for all QC DBS cards including Negative, Low, and High controls.
c. Enter these values into rows containing the QC DBS Cards
    i. Be sure to enter the values in the columns corresponding to the correct peptides.

7. Highlight all samples to be run and hit the play button in the toolbar to begin the analysis.
8. In the pop-up window, under operations select the following:
    a. Integrate Samples.
    b. Calibrate Standards
    c. Quantify Samples
9. Under the method dropdown menu select the correct TargetLynx Method.
10. Press OK.

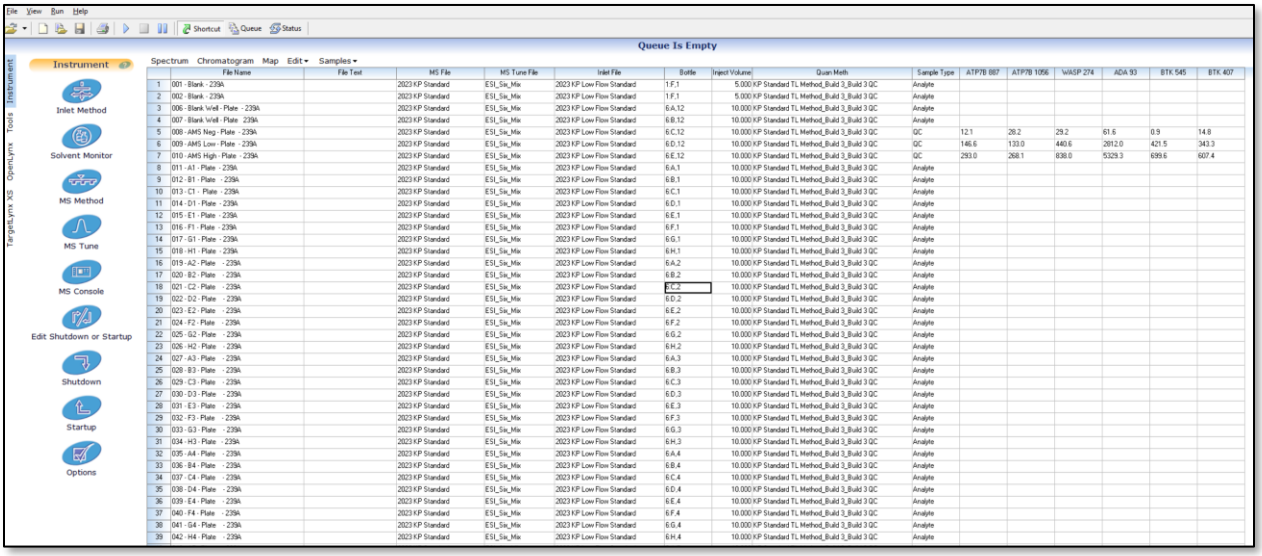


Figure 6. Example MassLynx Sample Layout

8.8. TargetLynx Results Procedure

1. On the left side of the MassLynx window, select “TargetLynx XS”.
    a. Select “Edit Method”.
    b. The Key Proteo Standard TargetLynx Method Editor should open.
    c. If it does not, navigate to File -> Open -> MethDB folder
        i. Select the Key Proteo Standard TargetLynx Method.

2. Set the lot specific Concentration Units for each peptide internal standard (**Figure 7**).
    a. There will be 12 compounds in the compound list.
        i. There will be an internal standard (IS) and an endogenous (light, L) compound for each of the six target peptides.
    b. Above the window on the right, select the “Calibration Properties” tab.
        i. For each IS compound in the compound list, there will be a lot-specific constant to enter under “Concentration of Standard”.
        ii. For each IS peptide, enter the constant value from the QC certificate next to “concentration of standard”.

1. This value incorporates blood volume, IS concentration, dilution, and lot specific calibration to generate a peptide concentration as an output.

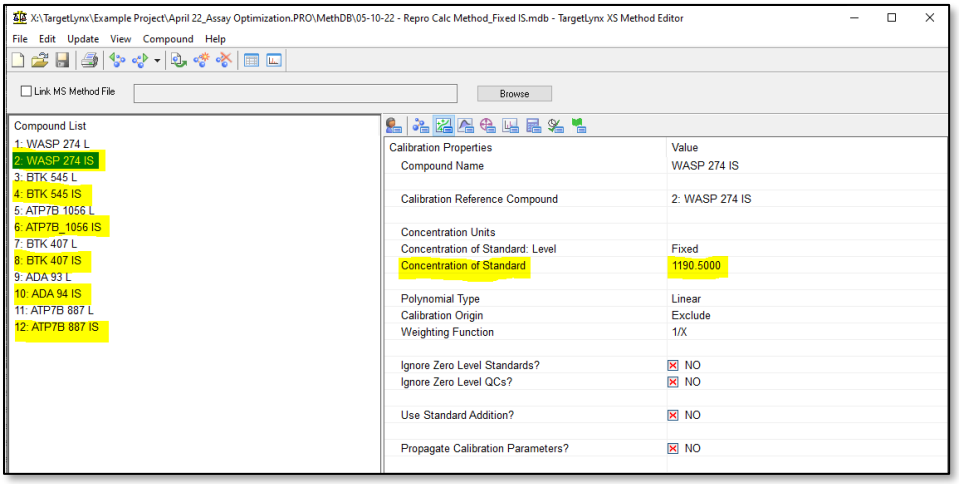


Figure 7. TargetLynx Concentration of Standard

3. Set the lot specific QC settings for each endogenous (Light) peptide (**Figure 8**).
    a. Above the window on the right of the TargetLynx XS method editor, navigate to the "QCMonitor" tab.
        i. For each L compound in the compound list, there will be a lot-specific QC and percent deviation values to enter under "QC Settings".
        ii. For each L peptide, enter the values from the QC certificate.
            1. These values set the allowable deviations from the pre-determined QC card concentrations and enable QC flagging.

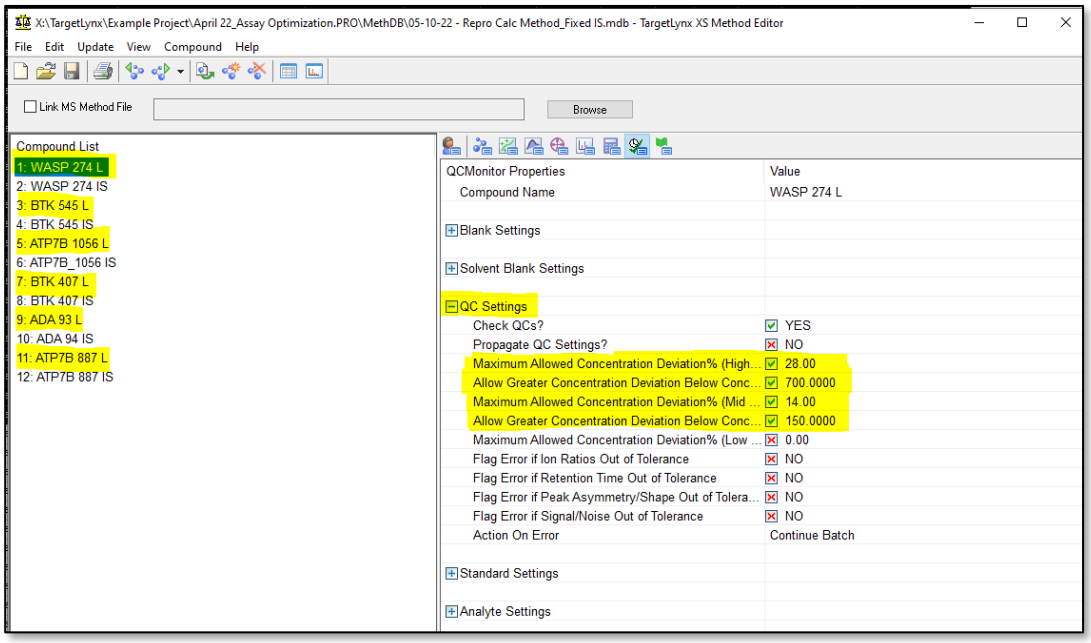


Figure 8. TargetLynx QC Monitor

4. TargetLynx method should now be prepared for sample processing.

8.9. Process Samples After Data Collection

1. Sample processing can be done after data collection.
    a. Open MassLynx software.
    b. Open the sample list that requires sample processing.
    c. Select all sample rows that require processing by clicking and dragging along the leftmost column.
    d. Highlight the “TargetLynx XS” tab along the leftmost side of the MassLynx window.
        i. Select “Process Samples” under the TargetLynx XS menu.
    e. A “Create TargetLynx XS Dataset” window will appear.
        i. Under operations select the following
            1. Integrate Samples
            2. Calibrate Standards
            3. Quantify Samples
        ii. Under the method dropdown menu select the “Key Proteo Processing Method”.
    f. Select OK.

8.10. TargetLynx Reports, Layouts, and Cutoffs

1. After processing, a TargetLynx Quantify MFC application window will open.

2. Two layouts will be provided that will format this TargetLynx window and allow for application of peptide cutoffs.
    a. Each layout will contain cutoffs for 3 peptides.
        i. KP_Layout 1_ATP7B_WASP contains cutoffs for ATP7B 887, ATP7B 1056, and WASP 274.
        ii. KP_Layout 2_ADA_BTK contains cutoffs for BTK 545, BTK 407, ADA 93.

3. Apply a Key Proteo layout.
    a. Select File -> Apply Layout
        i. A MethDB folder window will open.
        ii. Select one of the KP Peptide Layouts.

4. The Key Proteo Newborn Screening Reagent Kit Peptide Layouts will report Areas, Concentrations, Standard Concentrations, Deviations, Concentration Flags, and Cutoffs in columns.
    a. Chromatograms can be reviewed sample by sample.
    b. Deviations from QC concentrations can be monitored.

5. Peptide concentration cutoffs can be changed as needed (Figure 9).
    a. Load the TargetLynx layout that contains the peptide cutoff that needs to be changed.
        i. Right click the column containing the peptide of interest.
        ii. Select “Edit Column Properties”
        iii. Select “Formula”
        iv. A formula will be displayed of the format:
            1. Peak Response<=*[CUTOFF]*?1:0
        v. A formula will be preloaded with a recommended cutoff value e.g.

1. Peak Response<=100?1:0

vi. Changing the number between "=" and "?" will apply a new cutoff.
   1. Change this number
   2. Select "OK".

vii. The column will now flag any peptide with a concentration below the new cutoff with a "1".

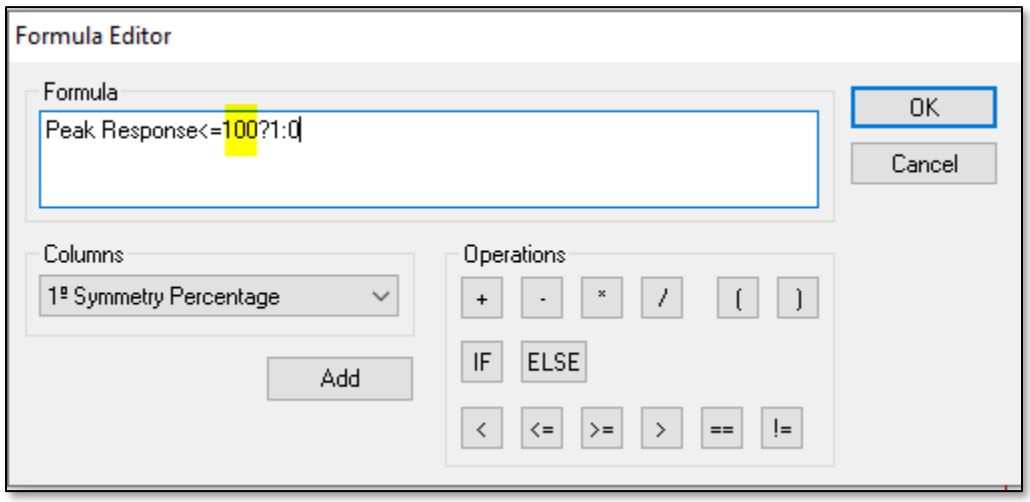


Figure 9: Setting TargetLynx Cutoffs

6. Each Layout can flag 3 cutoffs. Both layouts will be needed to monitor the six target peptides of interest.
7. To Add Concentrations that are generated.

8. Reports will need to be generated from both layouts.

8.11 . Procedural Notes:

- ***Internal standard plates, QC dried blood spots, and lot-specific QC reports are all lot specific and should not be mixed between lots.***

- Store and handle IS plates right-side up as lyophilized internal standard peptide pellets can become dislodged and lyophilized pellet dust can get stuck to adhesive of plate seal.
- The pellet itself can move around and potentially stick to adhesive.
  - If a pellet is loose in the well or stuck to the adhesive, clean forceps can be used to gently move the pellet to base of well to ensure accurate reading of sample.
- Ensure that all Wash Solution is removed from wells before Elution Solution is added.

10. Results and Reporting

10.1 Result Calculation: Target peptide concentrations are calculated using a ratio. The specimen peptide is determined as a specific concentration in the blood. Internal standard peptide is incorporated into the assay at a specific and known concentration. The ratio of the specimen peptide concentration to the internal standard peptide concentration gives the concentration of each peptide in the injected samples. This ratio is then multiplied by a constant value incorporating the assumed blood volume and between-lot calibration factors. Results are reported out in pmol/L of each peptide.

10.1. Run Failure:

10.1.1. Internal standard signals are monitored in wells A1 and A2 where no DBS samples are located. Lack of signal for any internal standard analyte in these wells constitutes a failure of the assay for that specific analyte. The sample run is recommended to be stopped and/or the samples are recommended to be repeated. The results are considered INVALID.

10.1.2. It is possible that an individual sample may experience a loss of chromatographic performance during a run. Any peptide signal presenting as a smear of signal across the gradient time or at a retention time different from the retention time of all other samples in the plate should be considered a failure and reported as INVALID and that specific sample should be re-tested. It is recommended that a third test is run to clarify the result.

10.1.3. Human blood is positive for all target peptides in the multiplex. The fish blood mixture is negative for all target peptides. DBS samples containing different levels of peptide targets will be created by serial dilution of NHC with negative fish blood. QC DBS cards will be processed twice during each run. These values for each individual peptide analyte at each of the low and high concentration levels should fall within the established ± 3 SD limits for the run to be accepted outright. Individual peptide values for the negative QC DBS cards should fall below pre-defined clinical cutoff concentrations to be accepted. Values outside of the established range across all QC samples on a plate will result in failure of a run and need to be retested.

10.1.4. Quality control DBS cards are supplied and must be processed alongside the assay on every plate. These standard deviation ranges are reported on lot specific quality control reports. QC DBS cards will be processed twice during each run. It is recommended that each lab establishes mean and SD values for each peptide and determine their own acceptable range of peptide values for QC samples. Control DBS results should be interpreted based on the laboratory's established criteria for acceptability and any state or federal requirements.

10.1.5. Presumptive positive results should only be reported from plates with passing DBS QC values as established by laboratory's internal guidance.

10.2. Values below LOQ: Values reported below the LOQ of the assay are positive for the targeted disease(s). Samples should be repeated to confirm peptide deficiency and reported as positive for the specific condition.

10.3. Values above Linear Range: Values reported above the linear range of the assay are likely negative for the targeted diseases and should be reported as negative (normal)

11. Quality Control

11.1. Materials: The quality control dried blood spots include 3 levels of control material: QC negative, QC low, and QC high. These spots contain mixtures of human and fish blood with varying concentrations of target peptides.

11.2. Use: It is recommended that QC samples are utilized to monitor the day-to-day validity of testing results. Control DBS at three levels are included in the kit. These controls should be run on each plate in accordance with the assay protocol. If more than one plate is run, these controls should be included on each plate. Peptide concentrations for all 6 target peptides are determined along with the newborn specimens. Lot-specific mean peptide concentration values and standard deviations (SD) will be provided with each Newborn Screening reagent kit. It is recommended that each lab establishes mean and SD values for each peptide and determines their own acceptable range of peptide values for QC samples. Patient results should be reported only if the control results meet the laboratory's established criteria for acceptability.

11.3. External Quality Control: QC testing should be performed in accordance to local, state and federal regulations. It is recommended to participate in external quality assurance programs such as CDC's NSQAP (Centers for Disease Control Newborn Screening Quality Assurance Program). It is also recommended that laboratories monitor trends in peptide concentrations for the QC samples on a weekly basis and compare them with the established limits.

12. Analytical Performance Characteristics:

12.1. Reproducibility/Precision: The precision studies were performed following the recommendations in the CLSI EP05-A3 guideline at three different laboratories. Repeatability (within-run precision), between-day precision, and reproducibility (between operators) were conducted utilizing a six level multi-analyte reproducibility panel (0,20,40,60,80, and100% human blood).

Study sites 1 and 2 were conducted over 5 days, with 2 plates per day and 3 replicate measurements of DBS samples with identical peptide concentrations by two operators for a total of 360 measurements. Each study site used 1 lot of reagents and 1 instrument.

Study site 3 was conducted over 20 days, with 2 plates per day and 3 replicates per plate by two operators for a total of 720 measurements.

Table 10. Site 1 Precision study (N=180)

Peptide	Panel	Mean (pmol/L)	Intra-day (%)	Between Day (%)	Between operator (%)
ADA 93	Panel A	379.6	26.8	24.2	35.2
	Panel B	900.8	6.1	11.2	10.4
	Panel C	1390.1	7.6	11.8	11.2
	Panel D	2263.5	20.8	24.4	24.4
	Panel E	2587.5	10.4	16.1	15.0
	Panel F	3324.0	8.2	14.8	14.0
ATP7B 1056	Panel A	NA	NA	NA	NA
	Panel B	59.1	5.1	8.5	7.9
	Panel C	79.2	11.8	12.9	12.3
	Panel D	117.5	9.9	11.3	10.9
	Panel E	156.0	6.0	9.7	9.5
	Panel F	223.0	9.3	14.6	13.5
ATP7B 887	Panel A	94.8	48.0	63.5	68.8
	Panel B	66.7	14.9	23.6	22.1
	Panel C	109.9	17.0	20.4	20.8
	Panel D	160.8	10.5	11.2	12.8
	Panel E	184.8	7.7	9.5	10.2
	Panel F	248.0	13.5	12.5	12.8
BTK 407	Panel A	53.9	6.8	10.3	10.1
	Panel B	107.3	21.7	23.5	25.5
	Panel C	192.3	34.5	35.2	35.9
	Panel D	268.2	12.5	17.5	18.9
	Panel E	335.3	21.6	21.8	21.8
	Panel F	405.0	11.6	10.9	11.0
BTK 545	Panel A	NA	NA	NA	NA
	Panel B	152.9	3.3	31.7	32.9
	Panel C	238.4	46.8	41.9	42.3
	Panel D	384.8	13.2	16.3	15.4
	Panel E	533.0	24.3	24.8	25.0
	Panel F	707.4	14.5	13.1	13.4
WASP 274	Panel A	190.1	19.3	21.8	20.8
	Panel B	188.7	7.4	34.9	31.4
	Panel C	226.1	9.8	35.3	31.8
	Panel D	279.9	11.0	26.0	24.0
	Panel E	324.5	10.8	26.5	24.1
	Panel F	380.9	10.7	17.1	15.9

Table 11. Site 2 precision study (N=180)

Peptide	Panel	Mean (pmol/L)	Intra-day (%)	Between Day (%)	Between operator (%)
ADA 93	Panel A	337.9	13.6	57.6	55.4
	Panel B	828.6	7.4	11.5	10.8
	Panel C	1387.8	8.2	9.7	9.2
	Panel D	2180.1	5.7	9.0	10.0
	Panel E	2854.8	6.5	9.3	9.5
	Panel F	3780.2	9.9	11.0	10.5
ATP7B 1056	Panel A	NA	NA	NA	NA
	Panel B	56.0	6.7	6.3	6.2
	Panel C	99.7	11.3	12.1	12.0
	Panel D	168.9	9.9	13.8	13.4
	Panel E	230.9	9.7	12.1	11.8
	Panel F	312.2	12.3	13.6	12.9
ATP7B 887	Panel A	NA	NA	NA	NA
	Panel B	56.8	9.7	11.2	10.6
	Panel C	101.0	13.9	13.8	14.1
	Panel D	154.6	8.3	10.9	10.6
	Panel E	200.5	8.8	11.1	10.6
	Panel F	246.7	11.1	11.0	10.9
BTK 407	Panel A	NA	NA	NA	NA
	Panel B	141.8	26.2	27.1	26.5
	Panel C	282.3	15.8	16.2	15.7
	Panel D	441.9	11.2	12.4	12.7
	Panel E	616.0	27.3	24.7	30.0
	Panel F	716.3	24.3	22.6	22.2
BTK 545	Panel A	NA	NA	NA	NA
	Panel B	145.5	21.1	21.0	21.6
	Panel C	299.8	20.0	20.3	19.7
	Panel D	497.1	15.7	16.9	16.7
	Panel E	728.9	28.7	26.9	30.5
	Panel F	865.2	22.7	21.4	20.9
WASP 274	Panel A	NA	NA	NA	NA
	Panel B	126.1	23.9	21.9	21.6
	Panel C	217.3	20.3	21.0	20.5
	Panel D	297.7	14.0	15.8	15.0
	Panel E	397.2	15.6	15.2	14.8
	Panel F	559.7	17.2	16.6	16.7

Table 10. Site 3 Precision study (N=720)

Peptide	Panel	Mean (pmol/L)	Intra-day (%)	Between Day (%)	Between operator (%)
ADA 93	Panel A	391.3	31.7	37.5	47.6
	Panel B	1066.2	22.0	36.0	33.7
	Panel C	1501.3	21.7	25.2	24.0
	Panel D	2224.6	18.7	24.0	25.1
	Panel E	2920.6	15.9	20.9	23.8
	Panel F	4178.2	20.2	31.0	34.6
ATP7B 1056	Panel A	68.0	NA	NA	NA
	Panel B	63.1	18.9	19.2	18.5
	Panel C	80.9	28.0	28.4	27.7
	Panel D	128.4	23.1	25.2	25.9
	Panel E	178.6	21.2	24.4	23.4
	Panel F	259.3	24.7	26.7	25.5
ATP7B 887	Panel A	71.3	36.8	37.8	38.1
	Panel B	92.4	33.0	35.6	37.3
	Panel C	123.7	24.9	26.9	28.1
	Panel D	156.9	20.8	25.5	25.9
	Panel E	197.0	21.5	22.5	21.8
	Panel F	260.6	20.6	23.3	22.3
BTK 407	Panel A	85.9	38.1	49.2	55.0
	Panel B	101.7	38.1	51.7	49.4
	Panel C	191.5	39.9	41.2	40.6
	Panel D	276.8	21.6	23.8	23.6
	Panel E	371.4	21.9	24.7	23.6
	Panel F	482.2	21.3	22.9	23.0
BTK 545	Panel A	166.0	NA	NA	NA
	Panel B	153.3	19.5	22.6	21.5
	Panel C	230.5	38.5	42.4	47.1
	Panel D	360.0	30.6	32.2	31.4
	Panel E	528.1	23.0	24.9	25.8
	Panel F	670.5	24.7	24.3	23.5
WASP 274	Panel A	83.5	50.4	48.3	50.3
	Panel B	114.8	30.1	40.7	38.0
	Panel C	181.8	32.0	33.6	32.2
	Panel D	244.4	21.8	22.6	23.5
	Panel E	321.2	22.6	26.9	26.1
	Panel F	402.7	24.8	26.2	25.7

12.2 Analytical Sensitivity: The limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ) for the test kit were determined following the recommendations in the CLSI EP17-A2 guideline with consultation of CLSI document C64 "Quantitative Measurement of Proteins and Peptides by Mass Spectrometry", involving replicate analysis of a set of blank and low-level samples using multiple lots and a single instrument.

The LOB, LOD and LOQ are summarized in **Table 13**.

Table 13. Analytical sensitivity

Detection Capability (pmol/L)	N	ATP7B 887	ATP7B 1056	WASP 274	ADA 93	BTK 407	BTK 545
Limit of Blank	120	15.5	13.7	6.2	77.5	7.5	2.6
Limit of Detection	120	24.5	22.7	23.9	172.7	21.4	13.3
Limit of Quantification	180	40.2	49.6	51.4	197.8	41.2	108.8

5 outliers were identified and removed them before determining the LOD and LOQ, as detailed below:

ATP7B 887: outlier A (z-score 2.4), C (z-score 2.5) and D (z-score 2.2)
ATP7B 1056: outlier A (z-score 2.0) and B (z-score -1.9)
WASP 274: outlier C (z-score 3.0)
BTK545: outlier E (z-score 3.5)
BTK407: outlier D (z-score 3.0) and E (z-score 3.2)

12.3 Linearity: The linearity study was performed following the recommendations in the CLSI EP06 guideline using two lots of reagents. The linearity of the assay was determined by replicate measurements of DBS samples with a range of endogenous peptide concentrations. These specimens consisted of fish blood and normal human control (NHC) blood in percentages from 0-100% NHC. For each peptide, the assay shows linearity for the intervals reported below, with deviations from linearity within 30%.

Table 14. Linearity range

Target Peptide	Linearity Range (pmol/L)
ATP7B 887	269.8-430.8
ATP7B 1056	70.3-451.9
WASP 274	51.4-547.0
ADA 93	197.8-3753.5
BTK 545	108.8-311.4
BTK 407	55.3-1290.7

12.4 Carry-Over: Carryover was determined by replicate measurements of blank samples with and without carryover for each multiplexed peptide**.** The blank samples were run followed by a DBS sample with high analyte concentrations for each multiplexed peptide and repeated to evaluate change in concentration due to carryover. The carryover effects of all peptides except ADA 93 were negligible. Based on the results, a specimen with ADA concentration within or slightly higher than the borderline cutoff should be retested if a specimen with ADA concentration within the normal range is present in the same column.

Table 15. Carry-over

Target Peptide	Carryover (%)
ATP7B 887	0.2-4.1
ATP7B 1056	0.9-4.2
WASP 274	0.1-0.6
ADA 93	5.0-21.2
BTK 545	0.4-2.0
BTK 407	0.0-0.8

12.5 Interference: Analytical specificity was determined in accordance with the CLSI EP7-A2 guideline. The interferents tested for the kit are as follows: Hematocrit, Unconjugated Bilirubin, Conjugated Bilirubin, Galactose, Glucose, EDTA, Heparin, Total Protein, Hemoglobin, and Triglyceride. Blood pools were made in both 100% fish blood and a 30% mixture of NHC and fish blood. Test pools were spiked with interfering substances. None of the 11 interferents tested in this experiment had a significant effect on the concentrations of all six peptides at the recommended initial interferent concentration levels (below 20%).

Table 16. Interference

Interference	Recommended Concentration	Unit
Unconjugated Bilirubin	0.4	mg/mL
Conjugated Bilirubin	0.4	mg/mL
Galactose	0.6	mg/mL
Glucose	10	mg/mL
EDTA	3.4	µM
Heparin	3.3	µg/mL
Total Protein	50	mg/mL
Hemoglobin	10	mg/mL
Cholesterol	400	mg/dL
Triglycerides	1500	mg/dL
Gamma-globulin	2.5	g/dL

The effect of hematocrit was tested by adjusting the amount of red blood cells to 40%, 50%, and 60% following the CLSI EP7-A2 guideline. The samples were constructed from mixtures of human and fish blood product. Human plasma and packed RBCs were mixed as described by CLSI guidelines NBS-04, Appendix F. 0%, 30%, and 100% human blood at 40/50/60% hematocrit was tested. The assay was not subject to interference for samples with low (40%) or high (60%) hematocrit with 30% human blood concentrations. However, the assay was subject to interference for five peptides (ATP7B 1056, ATP7B 887, ADA 93, BTK 407, and BTK 545) in samples in low hematocrit (40%) with 100% human blood concentration.

12.6 Outlier Rate: As detailed in section 11.4, a total of 5 outliers were observed in all the analytical studies from three sites (LOB/LOD/LOQ). Specifically, outliers were observed at a rate of 0.71% for ATP7B 887, 0.48% for ATP7B 1056 and BTK 407, and 0.24% for WASP 274 and BTK 545.

12.7 Stability: Stressed stability test was conducted in collaboration with Nelson Laboratories (Salt Lake City, UT). 3 kits were randomly selected from the manufactured lot and placed into controlled chambers. These kits were taken through a specified stress sequence (40 ˚C, -20˚C, 30˚C, and 2-8˚C) while the remaining the kits in the lot were stored at the recommended conditions of 2-8˚C. Temperature extremes affected ATP7B 887, ATP7B 1056, and WAS 274 concentrations.

The study was conducted to determine the stability of screening kit using a classical and real-time approach as described in the CLSI EP17-A2 guideline. In-use 30 days after open kit testing showed stable for up to 1 month, however the difference bias has increased at 6 months. Real time stability at 4˚C at 3 months and 6 months showed high bias likely due to degradation of IS plate. Frozen stability at -20˚C showed stable peptide concentration through 6 months with low CV and bias. We recommend storing the kit at -20˚C to prevent the internal standard from degrading over time and the properly stored kits are stable up to 6 months.

On-board stability testing was conducted by replicate panel of eluted samples placed into the instrument sample manager and measuring across time (0, 4, 8, 24, 32 and 48 hours). Differences in the six peptide concentrations across all time points were below 25%.

13 Key Proteo Newborn Screening Clinical Validation Study and Cutoff Determinations

12.1. Clinical Sensitivity
Not applicable

12.2. Clinical Specificity
Not applicable

12.3. Other Clinical Supportive Data

12.3.1. To establish the initial cutoff values, Key Proteo performed a preliminary study prior to the clinical study by analyzing 1,000 presumed normal de-identified samples and 12 positive samples. The cutoffs were selected to ensure that all known affected specimens would be detected and to minimize the false positive rate. The cut-off for ATP7B 887, 1056 was set for 25% of median values in pmol/L, 20% of median values in pmol/L for ADA 93 and the rest, BTK 407, 545 and WASP274 were set for 10% of median values in pmol/L. If the target peptide concentration was lower than cutoff, retest on remaining sample was performed. Only male newborns were reported for WAS and XLA test results. All positive samples were sequenced.

The initial cutoff used at the start of the study is shown in **Table 17**. The average peptide concentrations were not affected by the age of sample collection up to 7 days or gender for WASP274, BTK545 and BTK407.

Table 17. Initial proposed cutoff

	ATP7B 887	ATP7B 1056	WASP 274	ADA 93	BTK 545	BTK 407
Initial	66.6	67.5	226.4	1145.6	156.4	112.2

12.3.2. The screening performance of the kit was determined in a prospective clinical study of routine newborn screening samples and previously confirmed positive patient samples which have been saved in the biorepository at -20C for WD, XLA, WAS and ADAD. Six of them were original newborn blood spots from affected patients. A total of 3,294 newborns and 49 genetically confirmed positive samples were tested at three sites. No presumptive positive cases were detected at the three sites.

Table 18. Clinical study cohort

Site	NBS samples	Screen Negative	False Positive rate
1	601	601	0
2	601	601	0
3	2092	2092	0

Table 19. Blinded patient study cohort

Site	Patient samples	Screen Positive	False Negative rate
1	15	15	0
2	14	14	0
3	20	20	0

All confirmed 49 positive cases were screen positive, and repeats were concordant with initial results. Four confirmed Wilson disease cases had ATP7B 887 level above the cutoff while ATP7B 1056 level were below the cutoff. Two Wilson disease patients had ATP7B 1056 level above the cutoff but ATP7B 887 level were below the cutoff. In one confirmed case of Wilson disease, WAS 274 level was below cutoff. Sequencing showed the variant of uncertain significance.

Table 20. Confirmed patient cohort

Site	WD	XLA	WAS	ADAD	Total
1	10	2	2	1	15
2	9	3	1	1	14
3	14	3	2	1	20

12.3.3. To estimate the potential for false negative results of the test, the samples presumed to be normal were evaluated using next generation sequencing in a clinical molecular laboratory. Specimens included 20% or higher of the median values in pmol/L and were pulled from three different sites. (A total of 100 newborn samples).

ATP7B gene: Heterozygous genetic variants of uncertain significance (VUS) were detected in six routine samples and heterozygous pathogenic variants were detected in two routine samples that were screened negative. The second variants were not detected in those samples. In one routine case, two variants of uncertain significance were detected in which the screen was negative. The clinical significance is yet uncertain.

ADA gene: Three heterozygous variants of uncertain significance and one heterozygous pathogenic variant was detected that were screen negative. The second variants were not detected in all these cases.

BTK gene: One variant of uncertain significance was detected in a case that was screened negative. The clinical significance is yet uncertain.

12.4. Interpretation of Results

12.4.1. Applying cutoff and retesting

- Specimens with initial test results above the cutoff for all six peptides were presumed negative (or normal). No additional test was taken.
- Specimens below initial cutoff was retested and reported presumed positive when the target concentration was still below the cutoff.
- For XLA and WAS, the specimens from females were not reported regardless of the test results.

- The specimen in which two or more target peptides were below cutoff was considered potentially poor-quality sample.

13. **Expected Values and Interpretation of Results**

Please note that the values described in this insert should be used as a guideline only and each laboratory should establish their own cutoffs for their own populations.

- The measurement of target peptide concentrations for WD, XLA, WAS, and ADAD from the dried blood spots specimens is performed by using a cutoff concentration that distinguishes between presumed affected and presumed normal newborns. Each laboratory should follow its own procedures for establishing cutoffs. For the diseases targeted by the Newborn Screening kit, specimens with peptide concentrations below the cutoff are highly likely to have the specific diseases and should be referred for diagnostic follow-up.

- It is recommended that each laboratory establish cutoffs per laboratory guidelines and any applicable local, state, and/or federal requirements. It is recommended to include as many true positive samples as possible to estimate the cutoff values with higher confidence levels.

- The cutoffs should be selected to ensure that all known affected samples will be detected and to minimize the false positive rates, keeping in mind the expected incidence of the diseases.

- As larger numbers of samples are screened and presumed positive results are obtained, cutoffs should be reviewed in consultation with specialists who can provide additional guidance based on incidence rates, disease severity, and typical profiles of known positive patients. It is also recommended that the performance of the test be monitored for seasonal variability (for example by monitoring daily or weekly medians). If a laboratory chooses to apply a two-tiered cutoff, it is recommended that a borderline cutoff value for each analyte be set above the cutoff.

- Samples with results above the cutoff for all target peptides are considered low risk and should be presumed negative (normal). Local regulations and guidelines should be followed for the handling and reporting of presumed normal results. Samples that fall below the cutoff should be retested for confirmation. If the repeated sample results are different from the first result, repeat test for a third time for clarification.

14. **Appendices**

15. References

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Advancing Newborn Screening in Washington State: A Novel Multiplexed LC-MS/MS Proteomic Assay for Wilson Disease and Inborn Errors of Immunity

C. Klippel[1], S. Sandin[1], J. Park[1], M. Dutta[1], T. Winstone[2], X. Chen[2], D. Orton[2], A. Singh[3], J. Hill[3], T. Shahbal[3], B. Officer[3], E. Hamacher[3], J. Thompson[3], P. Duong[4], T. Grotzer[4], S. Hahn[1,4]

[1]Key Proteo, Inc., Seattle, WA; [2]Alberta Precision Laboratory, Calgary, Canada; [3]Newborn Screening Laboratory, Public Health Laboratories, Washington State Department of Health, Shoreline, WA; [4] University of Washington/Seattle Children's Hospital, Seattle, WA.

Newborn Screening (NBS) is considered one of the most successful public health programs in identifying infants with treatable disorders for early intervention with favorable outcomes. Unfortunately, for many genetic disorders there are no specific metabolic biomarkers nor any analytical methods suitable for population screening even where highly effective pre-emptive treatments are available. Most causative mutations in genetic disorders result in reduction or absence of their proteins, therefore, direct measurements of the peptide as a surrogate marker for these proteins using multiplexed proteomic methods from dried blood spots (DBS) can be highly diagnostic and utilized in population screening.

Direct measurement of signature peptides in DBS has been shown to be a sensitive and specific proteomic screening method for the multiplex detection of patients with Wilson Disease and three life-threatening inborn errors of immunity, X-linked agammaglobulinemia, Wiskott-Aldrich syndrome, and Adenosine Deaminase deficiency. Each of these disorders results in severe negative sequelae if undetected but are treatable if diagnosed early in life. Analysis of signature peptides found statistically significant reduction or absence of peptide levels in affected patients compared to control groups in each case.

A novel proteomic-based IVD (*Invitro* diagnostics) kit for NBS has been manufactured to identify these four conditions in a single-run multiplex assay from DBS with inject-to-inject time at < 3 minutes using LC-MS/MS. For the FDA De Novo Class II application, the screening performance of the kit was determined in a prospective clinical study of routine newborn samples and previously confirmed positive patient samples. In this validation study, a total of 3,294 newborns and 49 genetically confirmed positive samples were tested at three sites. No presumptive positive cases were detected, and all confirmed 49 positive cases were screen positive. Analytical performance including stability, day to day reproducibility, and inter-operator variation was all acceptable at three sites. A pilot study is also underway in conjunction with the WA State public health NBS laboratory. To date, more than 22,000 newborn samples have been screened, and no true presumptive positive cases have been found with only a few false positive cases detected. These ongoing studies support both the feasibility of newborn screening for these conditions and the use of multiplexed proteomic analysis as an effective methodology for pediatric population screening.

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Population screening for Wilson's disease

Si Houn Hahn

Department of Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle, Washington

Address for correspondence: Si Houn Hahn, M.D., Ph.D., Department of Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle, Washington. sihahn@uw.edu

Wilson's disease is an autosomal recessive disorder of copper transport caused by mutations in the gene encoding an ATPase, *ATP7B*. Early detection of Wilson's disease is critical because effective medical treatments such as chelating agents and zinc salts are available, which can prevent lifelong neurological disabilities and/or cirrhosis. It is unfortunate that most patients are brought to our attention after they have developed serious complications such as brain damage or cirrhosis, despite the availability of effective treatments. The diagnosis is usually made through copper measurement in the liver tissue, followed by confirmation with genetic testing of the *ATP7B* gene. Currently, there are no effective biomarkers or methods suitable for newborn screening for Wilson's disease. Ceruloplasmin has been tested for pediatric and newborn screening with limited outcome. Recently, liquid chromatography–multiple reaction monitoring–mass spectrometry (LC-MRM-MS) has emerged as a robust technology that may enable multiplex quantification of signature proteotypic peptides with low abundance. The application of this technology may help facilitate the research on Wilson's disease for protein expression, biomarker study, diagnosis, and, hopefully, screening.

Keywords: Wilson's disease; newborn screening; population screening; ceruloplasmin; ATP7B; LC-MS/MS; LC-MRM-MS

Wilson's disease has evolved from a diagnosable to a treatable disease over the 100 years since the first cases were reported in 1912 by S.A.K. Wilson.[1] The disease is progressive and ultimately fatal if untreated and is probably one of the most frequent causes of chronic liver disease in children. Although the prevalence varies across the population, it was estimated to be 1 in 30,000 with a carrier frequency of 1 in 90.[2] Over 500 mutations have been reported, along with >100 polymorphisms, in the *ATP7B* gene in the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ATP7B). The most common mutation in Europe and North America is p.H1069Q, while p.Arg778L is the most common in Far Eastern Asian populations.

Clinical and laboratory ascertainments for Wilson's disease are often very challenging, as clinical presentations of Wilson's disease show considerable variations and may be even much broader than we know. Guidelines for the diagnosis of Wilson's disease have been approved;[3] however, a small number of patients still cannot be diagnosed with current tests. Despite recent improved awareness and advanced DNA testing, a substantial number of patients still present with delayed diagnosis, which is unfortunate when we have effective treatments available that include copper chelators (trientine, penicillamine) to promote copper excretion from the body and zinc salts to inhibit the copper absorption from the gastrointestinal epithelium. Early diagnosis is critical for better prognosis. Patients with neurological symptoms may experience worsening with the treatment, and half of those patients may develop permanent neurological damage and will never return to the baseline of function.[4] Hepatic presentations range from acute and chronic hepatitis to cirrhosis and acute liver failure. Hepatocellular carcinoma has become an important recent issue for patients with Wilson's disease, as current treatment has improved life expectancy.

Considering all these factors, population screening for Wilson's disease has been extensively discussed, with strong needs; however, no screenable,

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Table 1. Results of population screening trials for Wilson's disease

Year	Study	No. of children	Age range	Positive cases	Method/sample
1993–1995	Yamaguchi *et al.*[7]	126,810	Newborns	0	ELISA, DBS
1977–1996	Yamaguchi *et al.*[7]	24,165	Late infancy to elementary school	3	ELISA, DBS
1999	Ohura *et al.*[8]	2789	1–6 years	2	ELISA, DBS
2001	Hahn *et al.*[11]	3667	3 months to 15 years	1	ELISA, DBS
2002	Owada *et al.*[9]	48,819	Primary school children	2	ELISA, urine
2006	Kroll *et al.*[12]	1380	Newborn to 18 years	0	ELISA, DBS
2008	Nakayama *et al.*[10]	11,362	3 years	1	ELISA, urine
2008	Zappu *et al.*[14]	5290	Newborn (Sardinia)	1:2707	PCR-based, DBS
		397	Newborn (Kalymnos)	27:20,000	

DBS, dried blood spots; ELISA, enzyme-linked immunosorbent assay.

cost-effective biomarkers or methods have yet been developed for Wilson's disease. To date, only a few studies have been conducted using ceruloplasmin as a marker, with limited outcomes (Table 1).

Newborn screening seeks to identify infants with treatable congenital disorders. Recent tandem mass spectrometry (MS/MS) applications have provided the ability to screen for >50 metabolic diseases from a single dried blood spot. The feature that makes metabolic disorders particularly amenable to screening is the presence of abundant small-molecule metabolites in plasma. However, many treatable disorders are characterized by absent or diminished large proteins in plasma or within circulating blood cells, for which there are currently no cost-effective screening methods. The research in our laboratory has focused on developing mass screening methods for these conditions, including Wilson's disease.

Previous experiences from pilot studies

Serum copper and ceruloplasmin are low in the majority of patients with highly elevated copper concentrations in the liver (>250 μg/g of tissue) and in the urine (>100 μg/day).[3] In some patients with Wilson's disease, serum ceruloplasmin is not low, and serum copper could be high in patients suffering from acute liver failure, owing to the release of copper from hepatocytes. Ceruloplasmin, a major copper-carrying protein in the blood, is a secretory enzyme that plays a role in iron metabolism as a ferroxidase. Ceruloplasmin is synthesized mainly by hepatocytes and incorporated with copper by the ATP7B protein before secretion into the blood. Earlier studies indicated that holoceruloplasmin (ceruloplasmin bound with copper) levels in plasma from patients with Wilson's disease were reduced, but the amount of apoprotein (ceruloplasmin without copper) was similar to that observed in normal individuals.[5] Based on this observation, a sandwich enzyme-linked immunosorbent assay (ELISA) kit was developed in Japan using a monoclonal antibody specific to holoceruloplasmin to measure the ceruloplasmin in dried blood spots.[6] Nevertheless, no patients with Wilson's disease were detected after screening over 126,810 newborns.[7] However, three patients were identified from 24,165 children from infancy to elementary school age in Japan. In another subsequent study using the same sandwich ELISA kit, two presymptomatic patients were identified among 2789 children aged from 1 to 6 years.[8] As a result of these studies, 3 years was considered to be the optimal age for Wilson's disease screening.

A mass screening measuring urinary holoceruloplasmin was also conducted in Japan.[9] The investigators found that the quantity of holoceruloplasmin protein in the urine of patients with Wilson's disease was significantly lower than in healthy subjects. However, in 2 of 41 Wilson's disease control samples (5%), urinary ceruloplasmin was not low, supporting previous observations that some patients with Wilson's disease could have normal ceruloplasmin levels. Two cases of Wilson's disease were found by testing urine samples from 48,819 primary school children. The rate of second request was about 0.9%. Another pilot study was conducted in Hokkaido Prefecture in Japan for 3-year-old children to screen for Wilson's disease using an automated assay of ceruloplasmin measurement in urine specimens.[10] A total of 11,362 children were screened, and one

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true positive case was identified and confirmed by genetic testing. The urinary ceruloplasmin level in Wilson's disease controls was 13 ± 9.25 ng/mg Cr, while the normal control was 190 ± 154 ng/mg Cr. When the cut-off value was 45.0 ng/mg Cr, the rate of second request was about 1% of total participants.

We also developed a sandwich ELISA method for ceruloplasmin measurement in dried blood spots using specific monoclonal antibodies against ceruloplasmin. In a small pilot study on 3667 children aged 3 months to 15 years, we identified one patient, a 32-month-old boy with a ceruloplasmin concentration of 2.3 mg/dL.[11] The rate of second request of this study was 0.3%. In a subsequent validation study, we were able to retrieve the original newborn blood spots from two affected patients with Wilson's disease along with age-matched controls.[12] The original newborn blood spots were kept in a –20 °C freezer. Ceruloplasmin concentrations in the original newborn blood spots from two patients were indeed very low: 2.6 and 2.8 mg/dL (normal control 47.2 ± 15.6 mg/dL with range from 6.5 to >60). It is conceivable that the ceruloplasmin concentration in Wilson's disease could be much lower during the newborn period compared to normal newborns, but further study will be required to determine the baseline level of ceruloplasmin in newborns with Wilson's disease. Although these findings supported the view that presymptomatic screening for Wilson's disease using dried blood spots could be feasible in the newborn period, subsequent screening over 100,000 newborn blood spots in Minnesota was unsuccessful in identifying a patient (unpublished data), similar to a previous experience in Japan.

We later developed an assay using liquid chromatography–triple quadrupole mass spectrometry (LC-MS/MS) to quantify ceruloplasmin-specific peptides from dried blood spot samples digested by trypsin.[13] Most state laboratories in the United States currently use LC-MS/MS for newborn screening. A method-comparison study on previously tested patient samples for ceruloplasmin gave comparable results, suggesting that it may be feasible to use LC-MS/MS for screening. Lower-limit quantification for ceruloplasmin was around 0.7 mg/dL. Inter- and intra-assay imprecision was acceptable for clinical use; however, this approach has not been studied in a large-scale population.

DNA analysis can be utilized for a genetically homogeneous group with highly prevalent mutations. In Sardinia, a 15-nucleotide deletion in the promoter region of *ATP7B* accounts for 61.7% of alleles. Six common mutations together account for approximately 85% of Wilson's disease in that population. By screening 5290 newborns in Sardinia, Wilson's disease incidence in this population was estimated to be approximately 1 in 2707 live births.[14]

Challenges

Ceruloplasmin is an acute-phase reactant, so the concentration can vary depending on the health status at the time of sample collections. Ceruloplasmin can be in the normal range in some symptomatic patients with Wilson's disease. Low ceruloplasmin can be observed in other conditions such as copper deficiency, Menkes disease, hereditary aceruloplasminemia, severe protein-losing enteropathy, or liver failure. Very little information is available for the baseline concentration of ceruloplasmin in Wilson's disease before patients develop symptoms.

The application of ELISA or immunoassay for ceruloplasmin quantification to newborns, regardless of different antibodies against ceruloplasmin epitopes, may not be sensitive enough to identify newborn patients with Wilson's disease. A substantial number of newborns present with physiologically low ceruloplasmin, which makes this approach difficult. The method using LC-MS/MS developed in our laboratory for ceruloplasmin measurement may also encounter similar problems with sensitivity and detection limit.

However, ceruloplasmin measurement by immunoassay, either on dried blood spots or urine specimens, could be a feasible approach for infants or older children. Indeed, pediatric or infant screening by ceruloplasmin assay seems the only amenable approach for screening Wilson's disease at present. The limited studies in Japan yielded some promising results. The cost is only $2.50 per test, but high false-positive rates, particularly for the urine test, necessitate considerable improvement of the assay. A disadvantage of this screening is that it would be effective only when used in conjunction with a mandatory health care program available at the age of 3 years. This system is not universally available worldwide and may be difficult to establish in the United States with limited federal resources.

Molecular genetic testing is useful in confirming the diagnosis in affected patients, but is also very useful in identifying affected siblings, including

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those without definite symptoms.[15,16] Although no single test can permit *de novo* diagnosis of Wilson's disease, the genetic test has been increasingly used in the clinical field and often underscores the important medical decision. Genetic tests play critical roles in the diagnosis of Wilson's disease. A stepwise approach with DNA-based screening in certain ethnic backgrounds or regions, starting with common mutations accounting for the majority of Wilson's disease patients in that region, may be an appropriate strategy.[17] Although the continuous decrease in the cost of DNA testing will render the screening more approachable, the current cost is still prohibitive for mass screening for Wilson's disease.

Recent investigation on 181 patients from the United Kingdom with clinically and biochemically confirmed Wilson's disease showed that overall mutation detection frequency was 98%.[18] The likelihood of mutation in genes other than *ATP7B* causing Wilson's disease is very low. Their study indicates that the frequency of heterozygosity is considerably higher than the previously reported occurrence of 1:90. The calculated frequency of individuals predicted to carry two pathogenic mutations was 1:7026, considerably higher than the reported prevalence of Wilson's disease of 1:30,000. This significant discrepancy between the genetic prevalence and the number of clinically diagnosed cases of Wilson's disease was explained by both a reduced penetrance of *ATP7B* mutations and a failure to diagnose patients. This underlines the need for more reliable biomarkers for screening Wilson's disease to prevent the development of serious complications.

As serum copper is reduced in Wilson's disease, measuring copper in dried blood spots was considered as a potential screening method. Unfortunately, copper is an environmentally abundant heavy metal; we detect very high copper content randomly in dried filter papers, which makes screening for Wilson's disease using dried blood spots unfeasible.

Future development

Many treatable congenital disorders are caused by mutations that result in absent or diminished levels of proteins; thus, protein biomarkers have enormous potential in the diagnosis/screening of congenital disorders. LC-MRM-MS has emerged as a robust technology that enables highly precise, specific, multiplex quantification of signature proteotypic peptides as stoichiometric surrogates of biomarker proteins. As proof of concept, we have developed a novel proteomic screening approach using LC-MRM-MS to simultaneously identify specific signature peptides derived from the transmembrane protein CD3ε (a general marker for T cell number) and the intracellular proteins WASP and BTK (expressed in B and myeloid cells) as markers of three life-threatening primary immunodeficient diseases (PIDD): severe combined immune deficiency (SCID), Wiskott–Aldrich syndrome (WAS), and X-linked agammaglobulinemia (XLA).[19] Blinded patients' peripheral blood mononuclear cell (PBMC) samples with sufficient actin recovery were successfully analyzed for BTK, WASP, and CD3ε signature peptides, using an actin peptide for normalization, to accurately identify patients with PIDD.

With depletion of high-abundance proteins, we were also able to identify signature peptides for BTK and WASP in the dried blood spot extract, along with their isotopically labeled standards. This is compelling evidence that detection of low-abundance proteins in dried blood spots will be feasible by employing specific enrichment techniques using antipeptide antibodies. Our lab is currently exploring the use of peptide immunoaffinity enrichment to overcome a number of caveats, including limited sensitivity and reproducibility, especially for low-abundance proteins or small sample volumes.[20,21] Our preliminary analysis indicates that candidate signature peptides for ATP7B in HepG2 cells can be identified, along with their isotopically labeled internal standards (unpublished data). A substantial proportion of Wilson's disease–associated missense mutations, including p.H1069Q and p.R778L, result in markedly decreased levels of the ATP7B protein caused by enhanced degradation.[22–24] Other prevalent mutations, such as protein-truncating nonsense mutations (~13% of known point mutations)[25] and frameshift mutations,[26] are predicted to result in the absence or decay of mRNA[27,28] or a severely truncated protein, resulting in absent or diminished protein levels. Taken together, it is expected that most patients with Wilson's disease would have absent or significantly reduced levels of ATP7B. Nevertheless, it is still not known whether the MRM-based technique could be sensitive enough or feasible for screening patients or newborns using blood-based specimens. The expression of ATP7B protein

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in blood-based specimens may not be high enough to be detected, even with this technology.

In summary, early recognition, diagnosis, and preemptive treatment are critical for improving outcomes in Wilson's disease. Wilson's disease meets the universal criteria for mass population screening, except that there is no cost-effective method yet available. Although the optimum time to screen is disputable, the newborn period has the advantage of an infrastructure that is currently in place for blood spots and screening worldwide. The current ceruloplasmin assay may be suitable for infant or pediatric screening around the age of 3 years, but there is a lack of outpatient-based mandatory programs, which is a significant barrier to implementation. A study using LC-MRM-MS technology is in progress, but at this point, no scientific data is available. New biomarkers and the development of new methods should be continuously explored to provide early and effective preemptive treatment that can drastically improve outcomes in patients with Wilson's disease.

Acknowledgments

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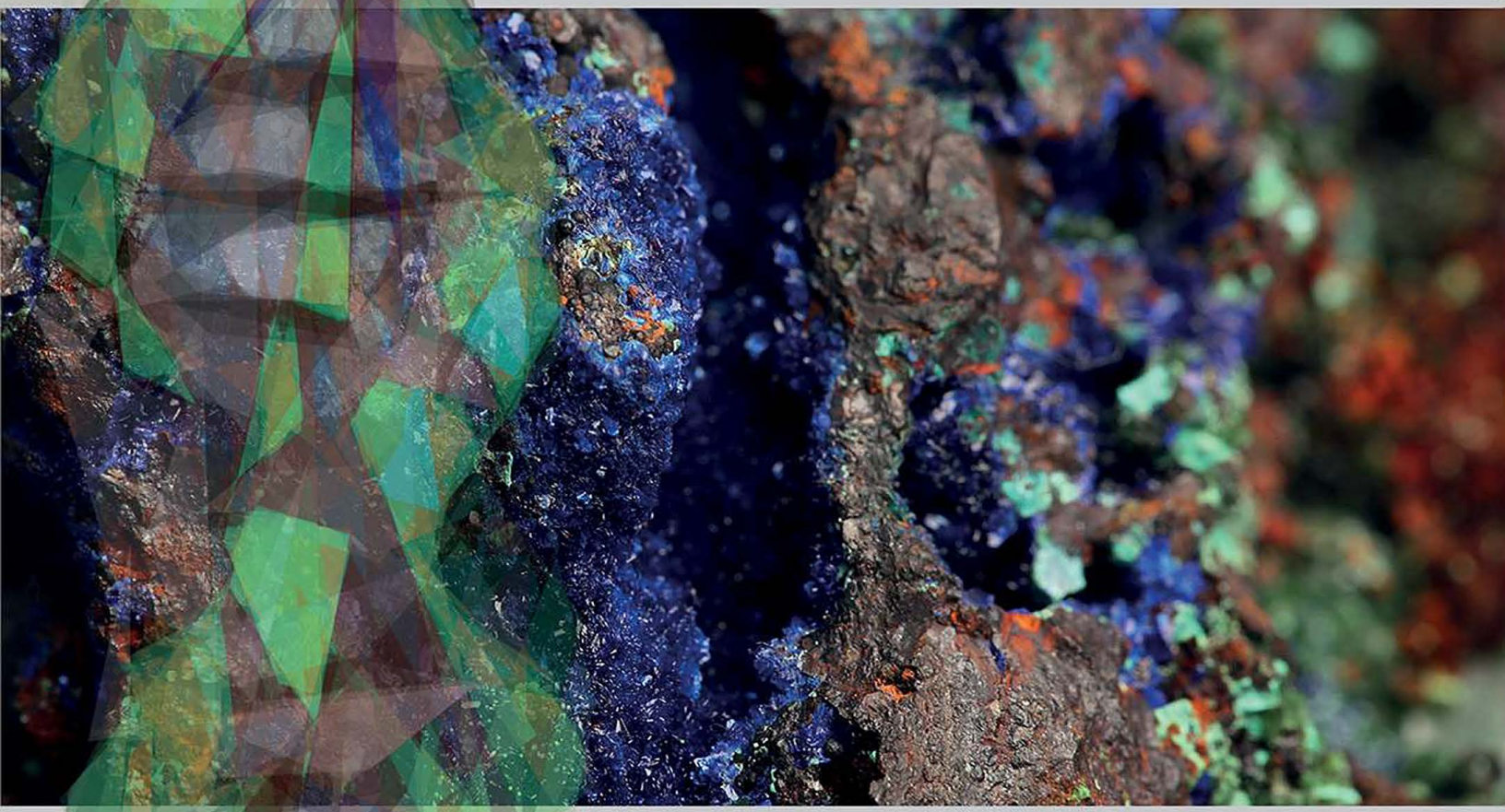
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Clinical and Translational Perspectives on WILSON DISEASE

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Chapter 26

Population Screening for Wilson Disease

Irene J. Chang[1], Sunhee Jung[2] and Si Houn Hahn[2,3]

[1]Department of Medical Genetics, University of Washington School of Medicine, Seattle, WA, United States, [2]Seattle Children's Hospital Research Institute, Seattle, WA, United States, [3]Department of Pediatrics, Division of Medical Genetics, University of Washington School of Medicine, Seattle, WA, United States

BACKGROUND

Wilson disease (WD, OMIM 277900) is an autosomal recessive disorder of copper metabolism with an estimated prevalence of one in 30,000 individuals and carrier frequency of one in 90 individuals in most populations [1]. The only gene known to be associated with WD is *ATP7B*, which encodes a copper-transporting ATPase ATP7B (Wilson ATPase) [2–5]. Over 500 different pathogenic variants and >100 polymorphisms have been identified in *ATP7B* in the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene = ATP7B), and most affected patients are compound heterozygotes, with one each of two different mutations [6]. There is wide geographic variation in *ATP7B* mutations [7–9]. These common mutations cause enhanced degradation and decreased levels of ATP7B, the ATPase abnormal in WD [10–13].

Effective treatment for WD is widely available and affordable, highlighting the importance of early detection and intervention. Copper chelators such as penicillamine and trientine bind excess copper and promote excretion, and zinc salts inhibit enteral copper absorption [14–18]. These treatments are highly effective in preventing the downstream morbidity and early mortality associated with WD [15–19]. Zinc monotherapy has also been demonstrated to be an effective long-term maintenance treatment for WD [20–24]. Left untreated, half of affected patients with neurologic symptoms may develop permanent neurologic damage [25]. Patients with chronic liver disease may progress to hepatocellular carcinoma [26,27]. Therefore, the ideal time to detect WD and begin treatment is prior to onset of symptoms for the best long-term clinical outcome.

NEWBORN SCREENING

Newborn screening is implemented by well-established public health programs in most developed countries. Each year, over 4 million newborn babies are screened for congenital disorders in the United States, and over 4000 infants are diagnosed as having a condition [28]. Given the incidence of WD, over 130 infants are likely born each year with WD.

Guidelines for deciding whether a particular condition is a suitable candidate for newborn screening was first formulated by Wilson and Jungner in 1968 and revised by the World Health Organization in 2008 [29,30]. Unfortunately, many aspects of these guidelines are subjective, and there is not always agreement about which disorders to include in the newborn screening panel [31]. Therefore, despite existing guidelines, there are now varying newborn screening panels worldwide. Nonetheless, given the aforementioned factors of high prevalence, severe disease burden, and available treatment, WD has always been an attractive target for newborn screening. However, no cost-effective, specific biomarkers or screening methods have been developed for WD. Previous studies have evaluated the predictive value of low ceruloplasmin, urinary copper, and holoceruloplasmin, with limited outcomes. The last decade has remained largely devoid of new studies on biochemical screening for WD.

The application of tandem mass spectrometry (MS/MS) has recently expanded the ability to screen for >50 metabolic diseases from a single dried blood spot collected on filter paper [32–36]. Inborn errors of metabolism are particularly amenable to screening due to the accumulation of small metabolites, which are detectable by mass spectrometry

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[37]. Many treatable disorders such as WD remain difficult to detect as they are characterized by absent or diminished levels of proteins or metabolites present only within leukocytes.

Recently, the emergence of liquid chromatography–selected reaction monitoring–mass spectrometry (LC–SRM–MS) has enabled multiplexed quantification of low-abundance signature proteotypic peptides [38,39]. The application of this technology may help facilitate research on WD for measuring protein abundance, identifying biomarkers, and enabling early diagnosis. Our laboratory has demonstrated a promising method of screening for WD by peptide immuno-SRM assay, raising the possibility of population screening for WD using peptide biomarkers for the ATP7B protein [40,41].

COPPER SCREENING

Most patients with WD have elevated serum non-ceruloplasmin-bound copper (>25 μg/dL), making serum copper quantification a logical potential target for screening in dried blood spots. Relative exchangeable copper (REC = exchangeable copper/total copper) has been proposed as a reliable, sensitive, and specific biomarker for screening and diagnosis of WD in adults (see Chapter 22: Direct Determination of Non-Ceruloplasmin-Bound Copper in Plasma). However, reference ranges have not been established for newborns or children [42]. Copper is also a naturally occurring abundant heavy metal in the environment. In a random sample of commercial filter papers, we detected very high copper concentrations, eliminating the possibility of screening for WD using any copper measurements in dried spots for both blood and urine.

CERULOPLASMIN SCREENING

Ceruloplasmin is an α_2-glycoprotein that contains 90% of circulating copper in the body [43]. Ceruloplasmin is initially synthesized in hepatocytes as apoceruloplasmin. Copper is then incorporated into apoceruloplasmin by the Wilson ATPase to form holoceruloplasmin before secretion into circulation. In WD, the absence of copper binding causes ceruloplasmin to be rapidly degraded by plasma proteases, leading to low plasma ceruloplasmin in most affected patients. Ceruloplasmin is an acute phase reactant, and the presence of low ceruloplasmin levels should be cautiously interpreted as other medical conditions may also lead to low ceruloplasmin (see Chapter 9: Ceruloplasmin). The majority of patients with WD have low plasma ceruloplasmin levels (<20 mg/dL or < 200 mg/L); however, some affected patients have been shown to have borderline or normal ceruloplasmin levels. Up to 20% of heterozygous carriers for WD may also have low-normal range serum ceruloplasmin levels [44]. Previous studies have demonstrated low diagnostic accuracy of serum ceruloplasmin for WD screening [45]. Using the conventional cutoff of 20 mg/dL, the estimated positive predictive value for WD was only 48.3% in adults with WD compared to carriers and controls [46]. A retrospective review of pediatric patients with hepatitis revealed accuracy of a ceruloplasmin level of <20 mg/mL of 84.8% [47].

Previous studies have demonstrated that while holoceruloplasmin levels were reduced in patients with WD, apoceruloplasmin levels were comparable to those observed in unaffected individuals [48]. Based on these findings, Endo and colleagues developed a sandwich enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody specific to holoceruloplasmin to measure ceruloplasmin levels in dried blood spots [49]. Their initial screening of 126,810 newborns did not detect any patients with WD, but their subsequent study of 24,165 infancy to elementary school-aged children identified three affected patients [50]. In another Japanese study using the same sandwich ELISA-based assay, two presymptomatic patients with WD were identified among 2789 children between 1 and 6 years old [51].

A pilot study of mass screening in 3667 children for WD in Korea using the sandwich ELISA assay identified one presymptomatic individual [52]. In a subsequent validation study, retrospective analysis of 353 newborn dried blood spots showed an average ceruloplasmin concentration of 40.0 ± 14.4 mg/dL [53]. Original newborn dried blood spots from two known affected patients were retrieved and ceruloplasmin concentrations were 2.6 and 2.8 mg/dL. A separate ceruloplasmin screening study on dried blood spots of ~100,000 newborns in Minnesota failed to detect any patients with WD (unpublished data). Ceruloplasmin levels in some newborns were unstable and physiologically lower than in older children and adults, and they continue to rise to adult levels 12 months after birth before stabilizing around 3 years of age [50]. Therefore, the optimal age of ceruloplasmin-based screening was thought to be ~3 years.

Other groups have studied population screening for WD by measuring urine holoceruloplasmin levels using sandwich ELISA assay. Owada and colleagues conducted mass screening of 48,819 primary school-aged children in Japan using urinary holoceruloplasmin and identified two presymptomatic individuals with WD [54]. Urinary holoceruloplasmin levels in the two affected patients were low at 1.9 and 9.3 ng/mg creatinine compared to 99.3 ± 77.7 ng/mg creatinine in unaffected 3–5-year-old children. However, two of the 41 WD control samples had higher levels of urinary

TABLE 26.1 Previous Population Screening Studies Using Ceruloplasmin Assays

Year	Study	Number of Subjects	Age Range	Positive Cases	CP in Positive Patients (mg/dL)	Mean CP (mg/dL)	PPV (%)/FP rate (%)	CP Cutoff Value	Methods/ Samples
1993–1995	Yamaguchi et al. [50]	126,810	Newborns	0	N/A	N/A	N/A	−2 to −2.5 SD	ELISA/DBS
1977–1996	Yamaguchi et al. [50]	24,165	Late infancy to elementary school	3	N/A	N/A (98.83% screened had CP > 20)	N/A	−2 to −2.5 SD	ELISA/DBS
1997	Cauza et al. [45]	2867	Adults	1 (then her sister)	N/A	±	PPV 5.9%	20 mg/dL	Radial immunodiffusion/ serum
1999	Ohura et al. [51]	2789	1–6 years	2	Pt1: 4.0,3.5 Pt2: 1.7,1.5	12.4 ± 3.95	FP rate 0.6%	3rd percentile	ELISA/DBS
2001	Hahn et al. [52]	3667	3 months to 15 years	1	2.3	30.5 ± 9.5	PPV 9.1%	15 mg/dL	ELISA/DBS
2002	Owada et al. [54]	48,819	Primary school children	2	Urinary HCP < 1.9, 9.3 ng/mg Cr, serum HCP 2.7, 7.0 mg/dL	99.3 ± 77.7 ng/ mg Cr Serum 21–37	N/A	3rd percentile	ELISA/urine
2006	Kroll et al. [53]	1398	Newborn to 18 years, retrospective	0	N/A, retrospective positive controls 2.6, 2.8	Newborn: 47.2 ± 15.5 Pediatric: 40.0 ± 14.4	N/A	< 15 mg/dL	ELISA/DBS
2008	Nakayama et al. [77]	11,362	3 years	1	13.3, 1.3, 24.9 ng/mg Cr	WD ctrl: 13.2 ± 9.25 ng/ mg Cr Normal ctrl: 190 ± 154ng/ mg Cr	N/A	1% of participants (<45.0 ng/mg Cr), 3 screenings	ELISA/urine
2008	Zappu et al. [70]	5290 397	Newborn (Sardinia) Newborn (Kalymnos)	1:2707 27:20,000	N/A	N/A	N/A	N/A	PCR-based/DBS

DBS, dried blood spots; *ELISA*, enzyme-linked immunosorbent assay; *CP*, ceruloplasmin; *HCP*, holoceruloplasmin; *Cr*, creatinine; *WD*, Wilson disease.; *N/A*, not available; *PPV*, positive predictive value; *FP*, false positive.

holoceruloplasmin within the range of normal controls, consistent with previous reports of patients with WD and normal ceruloplasmin levels. In addition, urinary holoceruloplasmin levels decreased after 3 days of storage in chlorhexidine gluconate at room temperature, and after 7 days at 4 °C. The accuracy and true predictive value were not reported. Furthermore, the study did not include phenotypic data or elaborate on the methodology behind which patients were selected for further testing. A follow-up study of urinary holoceruloplasmin levels in 11,362 primary school children in the island of Hokkaido identified one patient with WD, which was later confirmed molecularly. The wide range of normal values, uncertain accuracy, and instability for storage and transport make urinary holoceruloplasmin less plausible for newborn screening. Previous population screening studies using ceruloplasmin assays and polymerase chain reaction (PCR)-based mutation analysis are summarized in Table 26.1.

At present, assaying ceruloplasmin in late infancy or childhood appears to be the only cost-effective approach to population-based screening for WD. The studies in Japan had an average cost of USD $2.50 per assay, not adjusted for inflation. However, the childhood-based screening model relied on the mandatory healthcare program infrastructure in Japan, which is not universally available in other countries. In the United States, this program would be difficult to implement given limited federal resources, significant population influx and efflux, and lack of compulsory routine pediatric healthcare follow-ups.

From a technical standpoint, ELISA-based immunoassays for ceruloplasmin quantification in plasma and urine, regardless of type of antibody used against different ceruloplasmin epitopes, may not be sensitive enough to detect newborn patients with WD. Taken together, while most patients with WD appear to have low ceruloplasmin levels at baseline, the low positive predictive value, false positive rates in heterozygotes and normal newborns, and variable antibody binding makes ELISA-based assays less than ideal for population screening of WD.

MOLECULAR-BASED SCREENING

Previous studies have failed to detect biallelic pathogenic variants in *ATP7B* in up to 20% of patients with clinically diagnosed WD, raising suspicion for genetic heterogeneity [58]. However, it is more likely that novel variants, reduced penetrance, and unusual mechanisms of inheritance such as uniparental disomy or the presence of three concurrent mutations contribute to this discrepancy. Sequencing of the entire coding region and adjacent splice sites of *ATP7B* in 181 patients with WD in the United Kingdom showed an overall mutation detection frequency of 98%, strongly supporting monogenic disease [59]. The data also suggest a much higher prevalence of biallelic pathogenic variants in *ATP7B* than conventionally reported (that is 1:7026 instead of 1:30,000). Sequencing of all 21 exons of *ATP7B* is a time-consuming and cost-prohibitive approach to population screening for WD. However, targeted DNA analysis can be utilized in genetically homogenous populations with high prevalence of disease and allelic frequency for specific pathogenic variants. For instance, the incidence of WD is particularly high in Sardinia, where a molecular-based screening study of 5290 newborns estimated an incidence of one in 2707 live births [60]. In Sardinia, a 15-nucleotide deletion in the promoter region of *ATP7B* is implicated in 60.5% of patients with WD [61]. In aggregate, six common mutations constitute up to 85% of disease-causing alleles in that isolated population. In the Canary Islands, the L708P mutation is seen in up to 64% of patients [62].

In large, genetically heterogeneous populations such as China, the United States, and India, the most prevalent pathogenic variants have lower combined allelic frequencies; many affected individuals are compound heterozygotes for rare or novel mutations. In China, the two most prevalent mutations (that is, R778L and P992L) were present at a combined allelic frequency of approximately 40%–50% [57,63,64]. In Korea, the two most prevalent mutations (R778L and N1270S) account for 50% of cases [65]. In the United States and Europe, the allelic frequency of H1069Q accounts for approximately 30% of cases alone [8,66,67]. The allelic frequencies of common mutations in India vary largely by geographic region [55,56,68,69].

As the cost of molecular testing decreases and accessibility increases, a stepwise approach starting with DNA-based screening of common mutations accounting for the majority of cases in certain regions may become a feasible and rapid method for diagnosis. Indeed, Zappu and colleagues applied TaqMan technology to initially screen for the six most common mutations in Sardinia, followed by testing for the remaining 16 mutations in heterozygotes. They report a sensitivity of 94.6% with this stepwise screening approach [70]. Mak and colleagues demonstrated detection of 28 common mutations in WD patients in Hong Kong within 3 hours using a gene panel, real-time amplification refractory mutation system PCR, and green fluorescent dye for analysis [71]. In Taiwan, Lin and colleagues employed a three-tiered screening approach in 14 patients with WD and 50 normal controls. First, they screened for

the two most common mutations in exons 8 and 13 using High Resolution Melting analysis, followed by mutation analysis in exons 2, 5, 11, 12, 16, and 18 in negative cases, and finally examining the 5′UTR region and all remaining exons in twice-negative cases. Abnormal melting curves at each step were confirmed with direct DNA sequencing. They report that approximately 67.86% of *ATP7B* mutation alleles could be detected at the first step and 96.43% at the second step [72].

High-throughput technology, such as microarray chips, has also been examined for rapid, cost-effective screening of selective WD mutations [73,74]. However, the detection rate must be improved with follow-up direct sequencing. In China, a large-scale, two-step screening study based on array technology was recently conducted on 1222 patients with WD and 110 healthy controls [57]. Rapid multiplex PCR-MassArray was used to first screen for 110 mutational hotspots common in East Asian populations. Patients who were negative were analyzed with PCR-Sanger sequencing of all exons, flanking regions, and 5′UTR and 3′UTR regions. They identified 88 pathogenic variants and 9 novel mutations, with the six most common mutations accounting for 57.46% of all cases. The authors also report some genotype−phenotype correlation, the strongest being younger age of onset and lower levels of plasma ceruloplasmin and serum copper levels in patients with the R778L mutation. Tiered testing starting with array-based technology customized for common regional mutations offers a promising rapid and sensitive method for WD screening, but it cannot be implemented as a large-scale, universal screening method for WD due to the limited detection rate.

MASS SPECTROMETRY-BASED SCREENING

Liquid chromatography−MS/MS (LC−MS/MS) techniques enable antibody-independent, highly precise, and multiplexed quantification of signature proteolytic peptides as stoichiometric surrogates of biomarker proteins. There are multiple advantages to mass spectrometry-based screening methods on dried blood spots. First, the analysis can be performed on newborn dried blood spots, which are stable without any additional processing, modifications, refrigeration, or special transport. Second, the technology employs existing newborn screening infrastructure, which relies on mass spectrometers already present in most newborn state laboratories. Third, the method offers rapid turnaround. Fourth, the assay directly analyzes affected proteins with high specificity instead of screening for downstream accumulated metabolites. Most importantly, surrogate protein biomarker identification by mass spectrometry offers a plethora of opportunities for application to other treatable congenital disorders characterized by absent or low-abundance proteins such as lysosomal storage diseases and primary immunodeficiency syndromes.

More recently, our laboratory developed a novel proteomic screening assay using peptide immunoaffinity enrichment coupled to selected reaction monitoring (SRM) to improve the detection sensitivity of LC−MS/MS. SRM is a targeted mass spectrometry technique with increased sensitivity compared to profiling modes of analysis while maintaining high specificity for the target analyte and the possibility of multiplexing.

Our laboratory initially harnessed this technology to quantify ceruloplasmin by detecting its tryptic-digest signature peptides. After determining signature peptides, establishing internal standards, and conducting validation studies, our results showed patients with WD had lower ceruloplasmin levels by surrogate biomarker peptides compared to carriers and healthy controls. These findings were consistent with those obtained by ELISA assay, with average ceruloplasmin levels of 0−20 mg/dL in affected individuals, compared to >20 mg/dL in WD carriers. The lower limit quantification for ceruloplasmin was determined to be around 0.7 mg/dL, and inter- and intra-assay precision suggests feasibility for clinical use. However, large-scale studies using this technology have not been performed due to the relatively time-consuming sample preparation and long chromatographic run time [40].

As proof-of-concept, we used LC−SRM−MS to simultaneously identify signature peptides as surrogate biomarkers for three life-threatening primary immunodeficiency diseases—severe combined immune deficiency (SCID), Wiskott−Aldrich syndrome (WAS), and X-linked agammaglobulinemia (XLA). The signature peptides were derived from transmembrane protein CD3ε (a general marker for T-cell number) and intracellular proteins Wiskott−Aldrich syndrome protein (WASP) and Bruton tyrosine kinase (BTK) (expressed in B cells and myeloid cells), respectively [75].

Concentrations for each representative protein (that is, WAS, CD3ε, and BTK) were established in 45 control samples and signature isotopically labeled peptides were customized for each protein. In a blinded fashion, 16 de-identified peripheral blood monocyte samples with sufficient actin recovery from molecularly confirmed affected patients (specifically, 5 with WAS, 5 with SCID, and 5 with XLA) were analyzed for these signature peptides. All three signature peptide biomarkers were quantifiably detectable in unaffected leukocyte samples and absent in

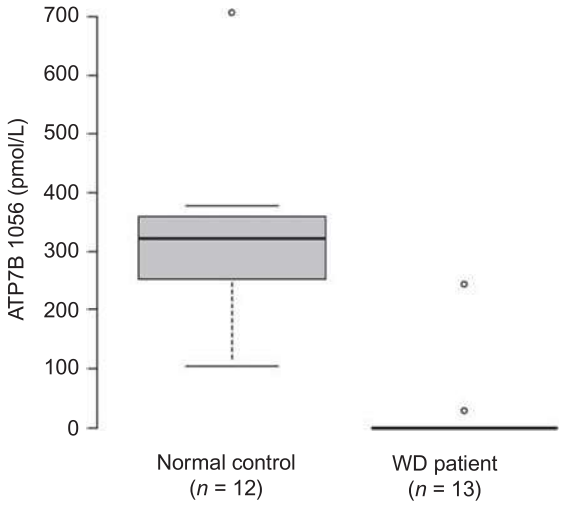


FIGURE 26.1 Distribution of the levels of ATP7B (Wilson ATPase) in dried blood spots from 13 WD patients and 12 normal controls. The bold black line indicates the median, the inner quantiles are represented by boxes, and the whiskers show 95% of the data. *WD*, Wilson disease. *Adapted from Jung S, Whiteaker JR, Zhao L, Yoo HW, Paulovich AG, Hahn SH. Quantification of ATP7B protein in dried blood spots by peptide immuno-SRM as a potential screen for Wilson's disease. J Proteome Res 2017;16:862–71.*

affected leukocyte samples using an actin peptide for normalization. The results were also replicated in T-cell depleted cell lines, demonstrating the ability of LC–SRM–MS to accurately identify patients with primary immunodeficiency disorders. However, the inter- and intra-assay coefficient of variation needed to be lowered for clinical application [75].

To improve the sensitivity and reproducibility of our results for low-abundance proteins and small sample volumes, we enhanced our assay with immunoaffinity enrichment using antipeptide antibodies coupled to SRM (immuno-SRM). Our most recent proof-of-concept study extended this technology to the detection of signature peptides to ATP7B protein in HepG2 cell lines and their isotopically labeled internal standards. This is based on the premise that many missense pathogenic variants associated with WD, including H1069Q and R778L, result in markedly decreased levels of ATP7B protein due to enhanced degradation. Other common WD mutations result in mRNA decay or severely truncated proteins, theoretically resulting in absent or diminished levels of ATP7B in leukocytes. Relative mRNA expression of ATP7B in various tissues, including peripheral leukocytes, has been shown in previous studies (http://biogps.org/#goto = genereport&id = 540) [76]. Therefore, we hypothesized that individuals affected with WD will have lower or absent signature peptides of ATP7B (Wilson ATPase) in their leukocytes and dried blood spots by immuno-SRM compared to carriers or negative controls.

Indeed, our study demonstrated reliable quantification of endogenous ATP7B biomarkers in dried blood spot samples by immuno-SRM. First, we used in silico trypsin digestion to select several signature peptides for ATP7B that are predicted to be well-detected by LC–MS/MS, using criteria previously described [75]. Next, affinity-purified rabbit polyclonal antibodies were generated against four signature peptides. The ATP7B 1056 peptide was selected as a target peptide for quantification in human samples based on good peptide recovery after antibody-enrichment, minimal background signal noise, and the fact that the most common WD mutation in Europeans, H1069Q, occurs within this peptide. Quantification of this ATP7B 1056 peptide was carried out by immuno-SRM on trypsinized dried blood spot samples of patients with WD and unaffected individuals. In 12 out of 13 samples from patients with WD, signature peptides to ATP7B were nearly absent compared to healthy controls, confirming our hypothesis (Fig. 26.1). The genotype and ATP7B 1056 levels in dried blood spots of the 13 WD patients compared to normal controls is depicted in Table 26.2. The one patient with ambiguous results by screening is a compound heterozygote with a variant of uncertain significance in *ATP7B*. Our findings provide compelling evidence that surrogate biomarkers of ATP7B protein may be used as a novel platform to screen for WD in dried blood spots [41]. However, whether the immuno-SRM-based technique is sensitive enough or feasible to screen newborn patients remains to be elucidated. Large-scale studies in newborns with proven carriers and controls are required in the future.

TABLE 26.2 Genotype and ATP7B Peptide 1056 Levels in Dried Blood Spots from 13 Wilson Disease Patients and 12 Normal Controls

Sample	ATP7B 1056 (pmol/L)	Mutation
WD1	29.5	p.R778W and p.T977M
WD2	ND[a]	p.H1069Q and p.R1319*
WD3	ND	p.H1069Q and p.R1319*
WD4	ND	p.G943D and p.T1178A
WD5	ND	p.R778L homozygote
WD6	ND	p.C2304_2305insc and p.L1083F
WD7	NA[b]	p.R778L and p.A874V
WD8	244.8	p.T974M and p.S391L
WD9	ND	p.R778G and p.K175S_fs/p.Q260P_fs
WD10	ND	p.R778G and p.K175S_fs/p.Q260P_fs
WD11	ND	p.R778L and p.E1064A
WD12	ND	p.R778L and p.E1064A
WD13	ND	p.H1069Q and p.Y1331Tfs*61
Controls ($N = 12$)	327.6 ± 147.4	

[a] *ND,* not detected
[b] *NA,* not applicable due to signal to noise ratio (S/N) < 10
Jung S, Whiteaker JR, Zhao L, Yoo HW, Paulovich AG, Hahn SH. Quantification of ATP7B protein in dried blood spots by peptide immuno-SRM as a potential screen for Wilson's disease. J Proteome Res 2017;16:862–71.

SUMMARY

Early detection and treatment of WD is imperative for optimizing long-term clinical outcomes. Diagnosis remains complicated by the nonspecific, broad phenotype of WD and occasionally ambiguous laboratory and molecular studies. WD meets consensus criteria for newborn screening, except that no reliable biomarker or cost-effective screening methods are currently available. Controversy exists on the optimal time to screen, as studies suggest ceruloplasmin assays are most sensitive and specific at around 3 years of age. However, the lack of mandatory outpatient-based pediatric health initiatives presents a challenge for widespread implementation in many countries such as the United States. Screening during the newborn period takes advantage of existing infrastructure for newborn dried blood spots and screening worldwide. Moreover, newborn dried blood spots are sufficiently stable, and they do not require any additional processing, modifications, refrigeration, or special transport. Initial studies utilizing LC–SRM–MS and immuno-SRM technology to quantify signature peptides to target proteins of various metabolic diseases show promising results, but larger studies in newborns compared to carriers and controls are needed. Research into novel biomarkers and honing of DNA- and mass spectrometry-based technology continue to push the frontiers of screening for patients with WD and may result in highly effective strategies for newborn screening.

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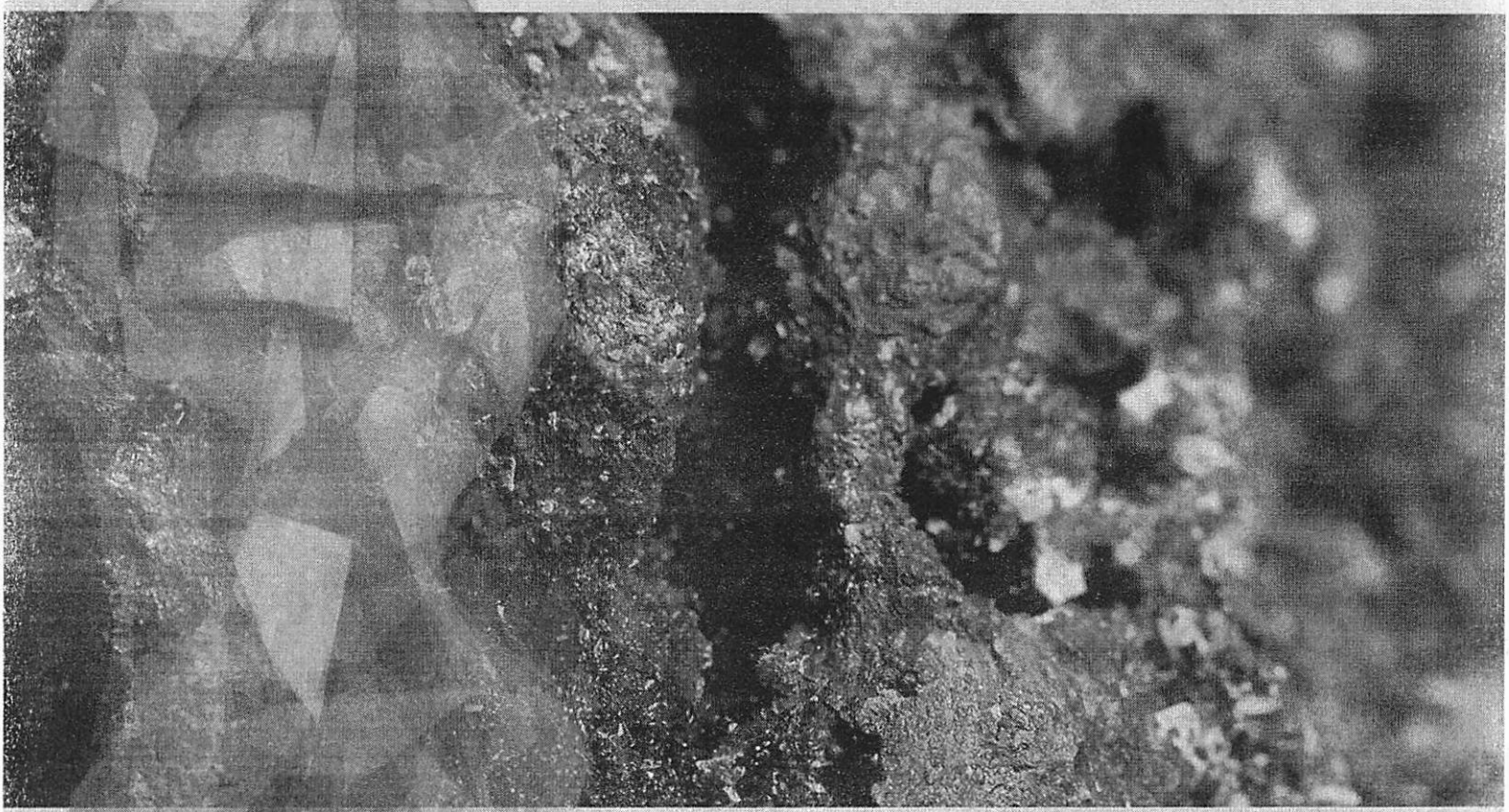
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Clinical and Translational Perspectives on WILSON DISEASE

Edited by
Nanda Kerkar
Eve A. Roberts



Chapter 17

Wilson Disease in Infancy through Adolescence

Nanda Kerkar[1,2] and Eve A. Roberts[3,4,5,6]

[1]*Division of Gastroenterology, Hepatology & Nutrition, Department of Pediatrics, Golisano Children's Hospital, University of Rochester Medical Center, Rochester, Rochester, United States,* [2]*Department of Pediatrics, University of Southern California, Los Angeles, Los Angeles, United States,* [3]*Department of Paediatrics, University of Toronto, Toronto, ON, Canada,* [4]*Department of Medicine, University of Toronto, Toronto, ON, Canada,* [5]*Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON, Canada,* [6]*History of Science and Technology Programme, University of King's College, Halifax, NS, Canada*

Wilson disease (WD), an inherited disorder mainly of hepatocellular copper disposition, due to dysfunction of the Wilson ATPase, a P_{1B}-ATPase encoded by the gene *ATP7B*, has been regarded as affecting mainly children and young adults. In children, as in older adult patients, WD has protean manifestations. It can involve multiple systems: notably hepatic, neurologic, and psychiatric but also ocular, hematological, renal, skeletal, and cardiac. An important general feature of WD in the pediatric age-bracket is that it often presents with nonspecific features which can be difficult to distinguish from other hepatic or neuropsychiatric disorders. A high level of clinical suspicion, along with effective methodical diagnostic testing, is required. Early diagnosis, preferably before development of clinical symptoms, is associated with normal, healthy life span in patients who are adherent to an effective medical regimen.

WILSON DISEASE IN VERY YOUNG CHILDREN

Classically, in the pediatric age-bracket, WD was most commonly diagnosed in adolescents. It is now well established that WD can present with clinical disease, mainly hepatic, in very young children. Definitions of "very young" children vary slightly among reports. We define "very young" as <5 years-old. Since the first detailed well-documented report of a 3 year-old with cirrhosis [1], numerous further reports have appeared [2–16], as shown in Table 17.1. Some children are diagnosed with WD when they present with an intercurrent health problem such as diarrhea and are found to have elevated serum aminotransferases; others are found through family or general infant screening. An early instance of molecular diagnosis in infancy was an 8-month-old Japanese boy picked up on mass screening [17]. A newborn infant was identified because of established genetic risk of having WD [18]. Among reports predating the identification of *ATP7B*, one appears to be actual WD, not simple copper toxicosis [19]. Many large case series include at least one child <5 years-old. Overall, there appear to be 50–60 such very young children currently reported in the literature. The majority have basal 24-hour urinary copper excretion >0.6 μmol/24 hours (>40 μg/24 hours); however, difficulties in collecting a 24-hour collection in this age-bracket can limit the test's utility. Finding elevated serum aminotransferases in a preschool-aged child is an indication to look for WD.

CLINICAL FEATURES IN CHILDREN AND ADOLESCENTS

The major clinical presentations of WD in children/adolescents are hepatic, neurologic, and psychiatric—and "silent" (no symptoms at all: apparently well). Occurrence of silent WD is extremely important in the pediatric age-bracket. Silent WD includes those patients conventionally described as being asymptomatic or having presymptomatic WD. These terms are often applied interchangeably, although they do not mean the same thing. (Calling a disease state "presymptomatic" makes the assumption that the disease will become clinically manifest.) Silent WD is generally identified

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TABLE 17.1 Features of Wilson Disease in the Very Young Child, Based on Available Published Data

Age, years	Number	Sex	Reason for Ascertainment	Serum Ceruloplasmin[a]	Basal 24-hour Urinary Cu[b]
0–1	7	5 M, 2 F	Incidental (diarrhea)→abnl LFTs: 2	No info: 2	No info: 3
			Family screening: 2; mass screening: 1; projected risk: 1; no info: 1	<100: 3	<0.6: 3
				>140: 2	>0.6: 1
1–2	6	1 M, 5 F	Family screening: 3; no info: 3	No info: 1	No info: 3
				<100: 4	<0.6: 3
				100–140: 1	
2–3	13	6 M, 7 F	Clinical liver disease: 1; "hepatic": 6	No info: 1	No info: 5
			Incidental→abnl LFTs: 3; no info: 3	<100: 4	<0.6: 2
				>140: 6	>0.6: 6
3–4	14	8 M, 6 F	Clinical liver disease: 1; "hepatic": 2	No info: 0	No info: 1
			Incidental→abnl LFTs: 3; pre-kindergarten exam→abnl LFTs: 3	<100: 7	<0.6: 3
			Family screening: 3; no info: 2	>140: 5	>0.6: 10
4–5	12	6 M, 6 F	Incidental→abnl LFTs: 2; family screening: 3; pre-kindergarten exam→abnl LFTs: 1; no info: 6	No info: 1	No info: 1
				<100: 8	<0.6: 1
				>140: 3	>0.6: 10
	52	26 M, 26 F			

abnl, abnormal; *LFTs*, liver function tests; *info*, information; *Cu*, copper.
[a] *< 100 mg/L suggests severe mutation; >140 mg/L is above suggested cutoff for ceruloplasmin in WD determined by immunological methodology.*
[b] *< 0.6 μmol/24 hours (<40 μg/24 hours) is below the informative threshold value for diagnosing WD in children.*

by screening or by chance. Individuals with silent WD have a definite diagnosis of WD, either genetically or biochemically (subnormal serum ceruloplasmin, basal 24-hour urinary copper excretion >0.6 μmol/24 hours; elevated hepatic copper concentration, if sought). While free of clinical symptoms, many have biochemical abnormalities indicating organ damage, typically, elevated serum aminotransferases.

Hepatic-WD tends to be evident at an earlier age than WD presenting with neurologic symptoms (called here "neuro-WD"); however, onset of neuro-WD or psychiatric manifestations of WD in childhood is not uncommon [20]. Psychiatric presentation may be very subtle. Based on data from numerous pediatric clinical series, gender distribution shows slight predominance of males over females (M:F = 5:4), whereas in adults, there may be a female predominance; average age at diagnosis is ~10 years-old.

LIVER DISEASE

The symptoms of hepatic-WD in children are frequently nonspecific, ranging from fatigue, anorexia, nausea, vomiting, weight loss to abdominal pain and jaundice. Jaundice is typical of more severe liver involvement but can be due to intravascular hemolysis. Jaundice may indicate liver decompensation in a chronic case or may be the presenting feature in classic Wilsonian acute liver failure (ALF-WD). If the rate of increase in serum bilirubin is high, the prognosis may be poor, as jaundice is then usually accompanied by other signs of liver failure including coagulopathy, ascites, and/or encephalopathy. Perhaps surprisingly, epistaxis is frequently mentioned in pediatric series as a presenting complaint. Hepatic-WD should be considered as a possible diagnosis in any child/adolescent who has hepatomegaly, elevated serum aminotransferases, or evidence of fatty liver.

WD is an important genetic cause of childhood cirrhosis worldwide. Actually determining the prevalence of cirrhosis in pediatric WD is difficult. A scan of the worldwide literature suggests that approximately one-third of children with clinically evident WD present with cirrhosis. The potential for rapid progression to cirrhosis emphasizes the

TABLE 17.2 Reported Clinical Features of Wilson Disease Resembling Autoimmune Hepatitis

		Age, Sex	Onset	IgG > ULN	Antibodies	Ceruloplasmin (mg/dL)	24-hour Urinary Copper (μg)	KF Ring/ DCT	Cirrhosis/ Interface Hepatitis	Liver Copper (μg/g)	Rx	Outcome
1	Milkiewicz [36]	15 years, F	Chronic	−	ANA 1:100, GPC +	1.1	213	−/−	+/+	385	Pred, D-Pen	Stable
2	Milkiewicz [36]	24 years, F	Chronic	+	SMA 1:60, ANA 1:40	1.8	326	−/−	+/+	965	Pred, LTx	Post-LTx
3	Yener [37]	22 years, F	ALF-WD	NA	ANA 1:400, SMA +	11.2	187.5	+/+	Necrosis	−	Pred	Death
4	Deutsch [38]	32 years, M	ALF-WD	+	ANA 1:1280	20	855	−/−	−/+	NA	Pred/AZA	Stable
5	Santos [39]	17 years, F	ALF-WD	−	↓C3, C4	14	10322	−/+	+/+	961	Pred, LTx	Post-LTx
6	Hunt [40]	29 years, M	Chronic	+	ANA 1:40	NA	NA	NA	+/−	1369	Trientine	Stable
7	Dara [41]	10 years, M	Acute Father: IDDM	−	ANA 1:160, SMA 1:80, LKM 1:20	2.8	1600 μg/dL	−/−	−/+	X20ULN	Pred/AZA and D-Pen	Stable
8	Loudianos [42]	15 years, F	ALF-WD	+	ANA 1:320, SMA 1:160	13.7	NA	+/−	+/+	388	Pred, D-Pen, LTx	Post-LTx

IgG, immunogloblin G; *DCT*, direct Coombs test; *KF*, Kayser–Fleischer ring; Rx treatment; *ANA*, anti-nuclear antibody; *GPC*, gastric parietal cell antibody; *SMA*, smooth muscle antibody; *ALF-WD*, classic Wilsonian acute liver failure; *Pred*, prednisolone or prednisone; *D-Pen*, D-penicillamine; *AZA*, azathioprine; *IDDM*, insulin-dependent diabetes mellitus; *LKM*, liver kidney microsomal antibody; *LTx*, liver transplant; *NA*, not applicable; +, positive/present; −, negative/absent.

importance of investigating persistent, even trivial, elevation of serum aminotransferases. In some countries, WD is an important cause of childhood cirrhosis [21,22]. Cirrhosis is uncommon in pediatric patients identified through screening of first-degree relatives [20].

WD can present as acute liver failure. When it is the initial presentation of WD, it may have highly characteristic clinical features. This classic Wilsonian acute liver failure (ALF-WD) typically consists of moderate-to-severe Coombs-negative acute intravascular hemolysis, coagulopathy unresponsive to vitamin K supplementation, some degree of encephalopathy including lethargy, relatively low serum aminotransferases running $\leq$2000 U/L throughout the clinical illness and very low, occasionally undetectable, serum alkaline phosphatase. Why alkaline phosphatase activity is severely depressed is uncertain but may be related to oxidative damage to the active site [23]. Renal failure attributed to renal tubular damage from copper may develop early in the course of illness. Jaundice may be severe, due to bilirubin load released from erythrocytes plus the renal insufficiency. Serum levels of copper not incorporated in ceruloplasmin are high, and consequently, urinary copper excretion is greatly increased. Classic Wilsonian acute liver failure occurs more often in females than in males and can occur in young children. It is worth noting that it is not unusual to see cirrhosis in the hepatic histology of a patient with ALF-WD either when liver biopsy is performed or in the explanted liver at the time of liver transplantation. Much less commonly, a less distinctive acute liver failure may occur with WD.

As obesity has emerged as a worldwide epidemic, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of liver disease in the pediatric population in the United States. The characteristic histology in NAFLD ranges from bland steatosis to steatohepatitis to cirrhosis [24]. Given that steatosis is also a characteristic histologic feature of WD, there is the possibility of missing a diagnosis of WD. A 4.5-year-old girl with fatty liver and nonspecific hepatitis on liver biopsy was diagnosed with WD based on copper studies. Her timely diagnosis was due to early liver biopsy whose histology led to appropriate WD screening [25]. While diffuse fatty infiltration is a known feature of WD [3,6,26], nodular fatty infiltration has also been reported in childhood WD [27]. Concurrence of metabolic syndrome and WD has been reported in adults [28,29]. While NAFLD is much more common than WD, failure to diagnose WD is serious. It is extremely important to rule out WD before labeling a child or adult with NAFLD as a diagnosis. The best test for distinguishing these two conditions is the basal 24-hour urinary copper excretion, which is much lower in NAFLD than in WD [30]. Checking serum ceruloplasmin is informative only if it reveals a distinctly subnormal serum ceruloplasmin, but the test is not very sensitive.

An important clinical presentation mainly in children, but also in young adults, closely resembles autoimmune hepatitis (AIH). This is a chronic inflammatory condition of the liver characterized by elevated serum aminotransferases, hypergammaglobulinemia, presence of nonspecific autoantibodies (anti-nuclear antibody, ANA; smooth muscle antibody, SMA; liver kidney microsomal antibody, LKM), interface hepatitis on liver histology, and response to immunosuppression. Diagnosis of AIH is facilitated by a scoring system, which has been revised and updated, since there is no single pathognomonic feature [31,32]. Classic AIH may have a chronic or an acute presentation. Importantly, the apparent simple acute hepatitis of WD may prove to be identical to an acute autoimmune hepatitis.

Since the early cases [33–35] of WD clinically resembling autoimmune hepatitis, numerous reports document a clinical presentation of pediatric WD resembling that of AIH (Table 17.2) [36–39,41,42]. In some, WD was misdiagnosed as AIH and treated, albeit inadequately, as AIH. For example, an adolescent girl with an 8-month history of jaundice, ascites, hyperglobulinemia, positive ANA, with interface hepatitis and established cirrhosis on liver biopsy, showed improvement with corticosteroid therapy, but a month later, WD screening results returned positive with low serum ceruloplasmin, increased urinary copper and liver copper of 385 μg/g dry weight. She was started on D-penicillamine and tapered off corticosteroids and became clinically stable [36]. ANA-positivity (type 1 AIH) is more frequent than LKM-positivity (type 2 AIH) in AIH-like WD but the latter pattern can occur.

Acute liver failure poses significant diagnostic problems since acute liver failure due to AIH can be difficult to diagnose. Pediatric patients with WD may present a similarly nondescript clinical picture of acute liver failure, not the classic picture of ALF-WD. A 17-year-old girl with acute liver failure was initially started on a trial of corticosteroids based on clinical and histological features of AIH, despite normal IgG and negative autoantibodies. Clinical deterioration prompted urgent liver transplantation. Histopathology of the explant was consistent with WD; her diagnostic basal 24-hour urinary copper excretion was 10,322 μg/24 hours (162.6 μmol/24 hours), results available only after the transplant [39]. A 15-year-old girl with acute liver failure had ANA 1:320, SMA 1:160, hypergammaglobulinemia, and plasma cell infiltrate on liver biopsy suggesting AIH, but she also had low serum ceruloplasmin, Kayser–Fleischer rings, and elevated liver copper concentration. She was started on both corticosteroids and penicillamine, but she underwent liver transplantation as her condition worsened. She had genetically proven WD [42].

A further problem is that possibly some individuals with WD may get autoimmune hepatitis as such. The disease mechanism in such cases might be similar to the situation where an individual with presymptomatic WD gets acute

liver failure from an intercurrent viral infection [43]. In this uncommon circumstance, the affected person develops AIH, possibly but not necessarily due to copper-mediated damage to the hepatocellular plasma membrane. Ulcerative colitis may occur in children who have WD with prominent autoimmune features [7,44]; however, it has also been reported as an adverse event from trientine [45]. Some children have a genetic makeup predisposing them to autoimmune disease: it might coincide with *ATP7B* mutations. In any case, occurrence of each disease needs to be established, or refuted, fully. Diagnostic problems may occur with primary sclerosing cholangitis [46] (Roberts EA, unpublished observations), or notably with autoimmune sclerosing cholangitis (ASC), because stainable copper may be found on liver biopsy. The pattern of distribution of copper in the liver tends to be different between these disorders and WD. In cholestasis, copper accumulation is typically limited to periportal hepatocytes. Early reports confounding WD and AIH may have been mistaking ASC for AIH, since ASC was not conceptualized until the 1990s.

NEUROLOGIC DISEASE

Neurologic disease is less common in pediatric patients with WD than in the adult age-bracket. It has been reported in children as young as 6 years-old [47] and rarely in preschool-aged children [48]. Walshe reported nine children with neuro-WD aged 12–19 years old [49]. A recent series included patients as young as 7 and 9 years old [50]. Neurologic features can develop in a child with hepatic-WD who is non-adherent to treatment. The neurologic changes of WD are predominantly extrapyramidal. Three distinct neurologic patterns have been described including dystonic, ataxic, and Parkinson-like syndromes, each with characteristic changes in magnetic resonance imaging (MRI) of the brain in each group [51]. The presence of "wing beating" or flapping tremor in association with dysarthria is characteristic of WD. "Risus sardonicus" or the involuntary grimace with an open mouth and contracted upper lip has also been described. These are classic and somewhat late findings. More commonly, in the pediatric population, subtle problems such behavioral changes, deterioration in handwriting, and inability to perform activities requiring good hand-eye coordination are noted. Pseudobulbar palsy can occur with either dystonia or ataxia: features include drooling, dysarthria, and problems with swallowing. Recent pediatric series feature dystonia, dysarthria, cognitive decline, tremor, gait abnormalities, and a Parkinson-like movement disorder, dysphagia, and drooling [50,52]. The majority of these pediatric patients with neuro-WD have Kayser–Fleischer rings. Seizures are atypical but may occur in some adolescents.

Diagnostic problems arise because of the subtlety of clinical presentation. Initial symptoms may be extremely nonspecific. One child presented initially with "learning disabilities" whose cause was discovered only in the course of investigating abdominal pain [53]. A teenager developed tremor, cramps in hands and feet, indecisiveness, and poor school performance [54]. Tremors, gait disorders, clumsiness, and school aversion may be too subtle to be noticed or may be attributed to teenage awkwardness.

PSYCHIATRIC DISORDERS

In Wilson's 1912 monograph, 8 of the 12 patients had psychiatric symptoms. Psychiatric symptoms can occur in both untreated and treated WD patients. Behavioral and psychiatric symptoms are more common in patients with neurologic involvement than in patients with hepatic involvement. Psychiatric symptoms may precede the recognition of hepatic or neurologic WD by a significant period of time, and there may be a delay in diagnosis of WD for several years [55,56]. This is likely because behavioral and psychiatric symptoms due to WD are often misdiagnosed. For example, they may be erroneously attributed to emotional stresses associated with puberty. The mechanism of development of psychiatric manifestations is not clear. Since many patients have the symptoms prior to diagnosis, it cannot be merely due to the psychological impact of the diagnosis. Other factors that have been explored are dopamine dysregulation from basal ganglia involvement, and the role of copper and trace elements in schizophrenia and bipolar illness [57,58]. Changes in circadian rhythm may be relevant [59].

The most common psychiatric features are abnormal behavior (typically increased irritability or disinhibition), personality changes, anxiety, and depression. Psychosis and schizophrenia have also been reported in WD [60,61]. Psychiatric presentation of WD may be more common in the pediatric age-bracket than is generally appreciated. Worsening performance at school or at work can be regarded as a psychiatric feature. A child whose academic performance drops off for no apparent reason requires consideration with respect to WD. Psychiatric aspects of WD in children presenting with hepatic-WD may not be prominent; moreover, development of psychiatric issues after the diagnosis of WD has received relatively little attention. It may have an impact on adherence (see Chapter 19: Wilson Disease: Psychiatric Aspects).

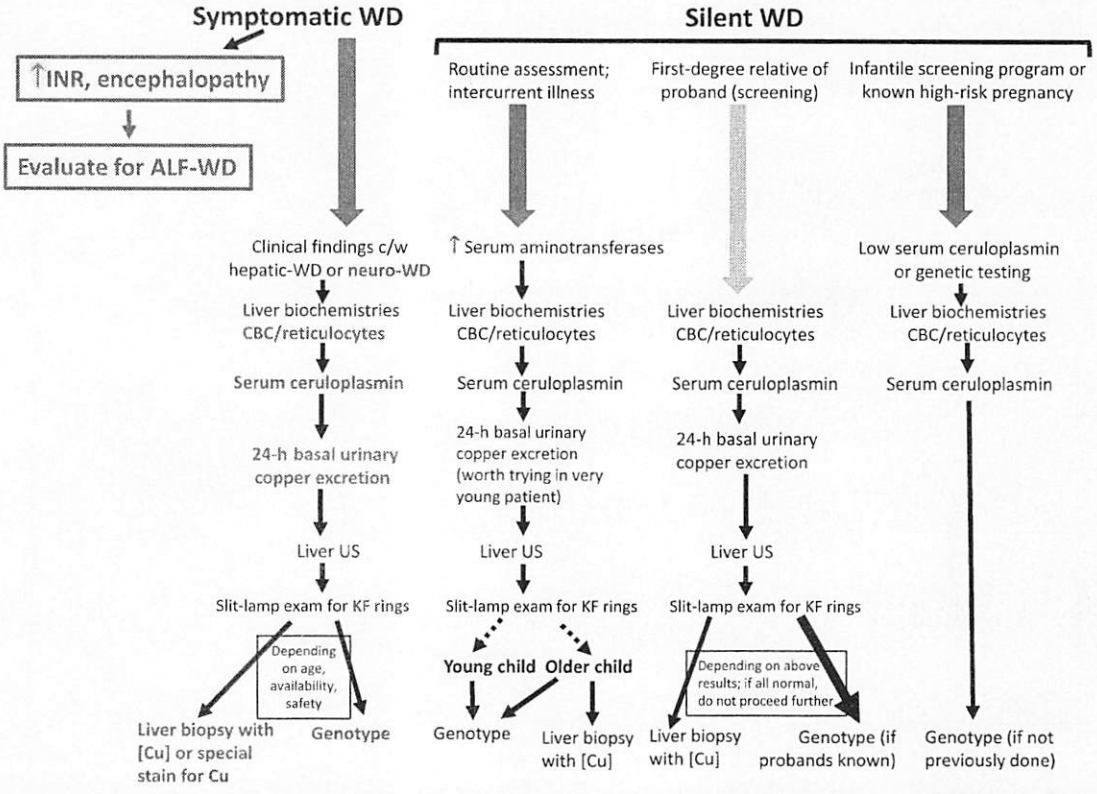


FIGURE 17.1 **A general approach to diagnosis of Wilson disease in the pediatric age-bracket, following comprehensive review of patient's medical history and a full physical examination.** Symptomatic WD comprises a spectrum of hepatic and neurologic disease: red lettering denotes inclusion in the Leipzig score for diagnosing WD. Silent WD includes children found incidentally to have nonspecific liver abnormalities (typically, elevated serum aminotransferases, measured as part of routine screening, or with an intercurrent illness) as well as infants/children identified through general population screening, based on ceruloplasmin or genetic testing. Silent WD also includes first-degree relatives of a WD patient for whom no preliminary data are available. "Liver biochemistries" signifies full liver panel including (at minimum) liver enzymes, total and conjugated bilirubin, albumin, and a coagulation test such as INR. Arrows indicate general progression of investigations, which can be grouped; dotted arrows indicate age dependence; heavy arrow indicates preferred investigation. *c/w*, consistent with; *24-h*, 24-hour; *US*, ultrasonography; *KF*, Kayser–Fleischer; *CBC*, complete blood count; *[Cu]*, parenchymal copper concentration; *ALF-WD*, classic Wilsonian acute liver failure.

OTHER CLINICAL FEATURES

Eye signs include the Kayser–Fleischer ring and sunflower cataracts. The latter are quite rare in children but were reported in a neurologically normal 8-year-old girl with WD [62]. Kayser–Fleischer rings are present in only ~40% of affected children. They are highly informative of the diagnosis when present. Slit-lamp examination by a skilled pediatric ophthalmologist is required for identifying them, especially in very young children. Neither Kayser–Fleischer rings nor sunflower cataracts affect vision. Both resolve with successful treatment.

Transient episodes of intravascular hemolysis are frequently reported in children, mainly as brief self-limited episodes of jaundice. Hemolysis may be an initial presentation of WD. In some cases presenting with hepatic or neurologic features, medical history reveals one or more previously undiagnosed hemolytic episodes. Hemolysis in WD is sometimes apparently precipitated by infection or drugs. A recent retrospective analysis of 321 WD patients revealed presentation with acute hemolysis in 22 (7%). The patients' age ranged from 7 to 20 years with an average onset of 12.6 years. Delayed diagnosis resulted in progression to severe hepatic disease and neurologic deterioration; 4 of these 22 patients may have had classic Wilsonian acute liver failure. [63]. Chronic hemolytic anemia may also occur in pediatric WD [64]. The mechanism is direct damage to the erythrocyte membrane by copper: hence, it is Coombs-negative. As previously noted, severe acute Coombs-negative intravascular hemolysis is prominent in classic Wilsonian acute liver failure.

Renal Fanconi syndrome is typical of WD in children. It may lead to nephrolithiasis. Renal tubular acidosis may also be present. In a Chinese series, five children aged 4–11 years old presented with a renal disorder causing pedal

edema [65]. Evident cardiac involvement is uncommon. Subtle asymptomatic myocardial dysfunction [66] or frank cardiomyopathy may be found [65].

Pediatric patients may have "osseomuscular" WD: arthralgia or less commonly arthritis, proximal muscle weakness, bony deformities, and pathological fractures [67]. These patients are mainly from the Indian subcontinent. Osseomuscular WD seems to be less common elsewhere. Individual musculoskeletal involvement including arthritis, myopathy [68,69], noteworthy muscle cramps [54,70], and rhabdomyolysis [71] has been reported. Osteochondritis dissecans have also been reported [72]. Diagnosis can be delayed if these complaints are misattributed: for example, confused with benign "growing pains" [73].

Other relatively rare organ involvement includes hypoparathyroidism and pancreatitis [74]. Skin signs in children are nonspecific but include extremely dry skin [75]. A clinical picture resembling Henoch–Schönlein purpura with palpable purpura was reported in pediatric WD [65].

DIAGNOSIS

Diagnosis of WD in children can be difficult (Fig. 17.1). The problems of diagnosing WD in a child with acute liver failure may be mitigated by finding Coombs-negative acute intravascular hemolysis, cholestasis in combination with a strikingly low serum alkaline phosphatase, and comparatively low serum aminotransferases from the onset of clinical illness. Very low serum alkaline phosphatase may also be found in WD with severe liver disease not quite qualifying as acute liver failure. For the child with less severe liver disease, such as seronegative acute hepatitis or unexplained elevation of serum aminotransferases or unexplained hemolysis producing jaundice, the most important biochemical criteria are serum ceruloplasmin and basal 24-hour urinary copper excretion, which is an indirect quantification of non-ceruloplasmin-bound copper in the plasma compartment. Measurement of hepatic parenchymal copper concentration provides critical data but involves an invasive procedure. For most patients, genotype determination demonstrating homozygosity for one disease-causing *ATP7B* alteration, or compound heterozygosity for two, is conclusive of the diagnosis. Genotype interpretation can be complex (see Chapter 14: The *ATP7B* Gene).

Among biochemical tests, serum ceruloplasmin remains an important parameter, despite being insufficient as a single test to rule in or exclude WD. Basically, the immunologically based analytical methods for measuring serum ceruloplasmin now in general use for automated measurement of serum ceruloplasmin have changed how that parameter supports the diagnosis of WD. Immunological measurement of serum ceruloplasmin measures apo- as well as holo- (copper-containing) ceruloplasmin. Fully functional holoceruloplasmin is produced as a function of the Wilson ATPase itself; its half-life is ~5.5 days. In contrast, apoceruloplasmin has a relatively short half-life of about 5 hours [76]: serum levels tend to end up lower than normal. Levels may be exceedingly low if the mutation is truncating. Thus, very low serum ceruloplasmin (<50 mg/L, or <5 mg/dL, measured immunologically) may be taken as strong evidence in favor of the diagnosis of WD. One clinical approach is to regard 140 mg/L (14 mg/dL) as the informative cutoff [77]. A more pragmatic approach is to investigate anyone with a subnormal serum ceruloplasmin. As many as one-third of pediatric WD patients may have a normal serum ceruloplasmin. In children with congenital nephrotic syndrome, serum ceruloplasmin may be subnormal due to excessive urinary loss, but anemia is present, as well as features of renal dysfunction [78]. Serum ceruloplasmin level is elevated by inflammation or the oral contraceptive pill since ceruloplasmin is an acute phase reactant.

Measurement of the basal 24-hour urinary copper excretion is valuable. Urine must be collected in copper-free containers; completeness of a full 24-hour collection should be confirmed by measuring urinary creatinine excretion (normal: 10–20 mg in 24 hours). The reference value of 100 μg/24 hours (1.6 μmol/24 hours) is insensitive for diagnosing WD in any age group [79]. The value >0.6 μmol/24 hours (>40 μg/24 hours) is preferable for children. This recommendation is based on a series of 29 pediatric patients of whom 27 had basal 24-hour urinary copper excretion exceeding 0.6 μmol/24 hours (>40 μg/24 hours); in that series, 8 of 29 (28%) had values <1.6 μmol/24 hours (<100 μg/24 hours) [26]. Systematic assessment by ROC analysis showed that 0.6 μmol/24 hours (40 μg/24 hours) is the best cutoff (sensitivity = 79%; specificity = 88%), at least in children [8]. In this latter study, patients who did not reach this level of cupruria were girls under the age of 4 years-old. Clearly, this test is limited by potential difficulties in getting an accurate 24-hour urine collection in very young children. Otherwise, it has physiological rationale and works well. For the purposes of screening, erring toward false positives is acceptable. Chronic cholestatic disorders such as MDR3 deficiency causing chronic cholestasis [80] can produce cupruria >0.6 μmol/24 hours (>40 μg/24 hours): genetic diagnosis is required.

The penicillamine challenge test, in which 500 mg D-penicillamine is given by mouth as a 24-hour urine collection is commenced and then again 12 hours later at the halfway point of the collection, was recommended for diagnosing

WD in children [81]. The diagnosis was established if urinary copper excretion equaled/exceeded 25 μmol/24-hours (>1600 μg/24-hours). Over time, this test has proved less reliable. In various series, 40%–50% or more of patients failed to meet that diagnostic criterion and yet were shown to have WD. It appears that asymptomatic patients may not be detected [82], possibly because the test was developed based on findings in WD children with established disease. The practical problems of testing very young children apply, and the best dose of D-penicillamine for testing very young children is not determined.

Liver biopsy can be highly informative for the diagnosis of WD in children, but it immediately entails the risk and cost of an invasive procedure. The time-honored criterion of hepatic parenchymal copper concentration >250 μg/g dry weight as diagnostic for WD is actually based on an early small study. Thus, the criterion has been subject to reassessment [30,83]. For children with WD, the proposed threshold of 75 μg/g dry weight requires further evaluation: thus, the cutoff of >250 μg/g dry weight remains preferred. Further diagnostic testing is required for children whose hepatic parenchymal copper is between 75 and 250 μg/g dry weight. Some risk of misdiagnosis of WD exists when liver copper content is used as the only test [84]. While normal hepatic parenchymal copper concentration (<50 μg/g dry weight) argues against the diagnosis of WD in the symptomatic patient, erroneously low concentrations can be found if the sample is too small. Presence of cirrhosis may also compromise this test. Sometimes hepatic copper content is not dramatically increased in toddlers with WD. Likewise, in ALF-WD, hepatic parenchymal copper shown by special stains may be misleadingly low.

Identifying an individual as homozygous for a disease-causing *ATP7B* mutation establishes the diagnosis of WD. Many individuals with WD have one each of two different disease-causing mutations. More complex patterns of inheritance may occur (see Chapter 14: The *ATP7B* Gene). In the context of first-degree-relative screening, if the genotype of the proband is known, genetic testing is the most efficient method for identifying siblings with WD. (Genetic screening of a pediatric proband's offspring may be more complicated—at least, logistically.) Generally in children, genetic testing is invaluable for diagnosing cases with atypical clinical findings because it provides the definitive diagnosis.

Scoring systems fall broadly into two categories: for diagnosis and for estimating prognosis (see Chapter 25: Role of Scoring Systems in Wilson Disease). The Leipzig criteria developed for adult patients [85,86] have been modified for use in the pediatric age-bracket [8]. A problem in the pediatric age-bracket is that often genotype is the best, or only, route to a Leipzig score diagnostic of WD. A methodical approach to diagnosis of WD in children may be the best strategy, as opposed to depending on a scoring system (see Fig. 17.1). Among scoring systems for predicting prognosis, the modified King's College Hospital score (new Wilson Index) is in wide use [87] (see Chapter 25: Role of Scoring Systems in Wilson Disease).

WILSON DISEASE-MIMICS

At the present time, mutations in the gene *ATP7B* appear to be the only genetic etiopathogenesis of WD. However, a few disorders have some claim to being a WD-mimic: namely, a different genetic defect produces a highly similar disorder. These include MEDNIK syndrome (mental retardation–enteropathy–deafness–neuropathy–ichthyosis–keratodermia), certain congenital disorders of glycosylation, and a hepatic disorder characterized by manganese retention in hepatocytes (see Chapter 41: Disorders that Mimic Wilson Disease). Indian Childhood Cirrhosis and similar infantile copper toxicoses (see Chapter 44: Indian Childhood Cirrhosis and Other Disorders of Copper Handling) feature hepatic copper accumulation but can be distinguished clinically.

SCREENING NEWBORNS AND PEDIATRIC FIRST-DEGREE RELATIVES

WD fits the criteria of a disease suitable for general newborn and infant screening; however, finding reliable and cost-effective methodology for such screening has proven challenging (see Chapter 26: Population Screening for Wilson Disease). A sensitive immunoaffinity enrichment mass spectrometry test to quantify Wilson ATPase in dried blood spots of newborns may serve effectively to screen for WD [88]

Retrospective reviews have shown that screening brothers and sisters of a child diagnosed with WD and initiating treatment in those found to have WD before they manifest symptomatic disease prevent clinical WD [89,90]. Screening involves physical examination, liver biochemistries, serum ceruloplasmin, and basal 24-hour urinary copper excretion. If positive, liver biopsy with measurement of parenchymal copper should be performed. If the genotype of the proband is known, genetic testing of first-degree relatives is an efficient option, when available and affordable (cost covered by insurance for those who cannot pay). A problem for managing these patients is that they may not fully understand that they have a serious disease: adherence to treatment may be poor [89,91,92] (see Chapter 37: Transition of Care and Adherence in Patients with Wilson Disease).

PROGNOSIS

Early diagnosis, preferably before a child/adolescent has symptomatic hepatic or neurologic disease, is most likely to result in near-normal longevity with generally good health so long as the patient tolerates effective medication, is adherent to the lifelong treatment regimen, and has consistent access to the medication. Families/Patients need to understand that stopping therapy completely may be fatal. An urgent contemporary social justice problem for children with WD is to assure life-long accessibility to effective drug treatment.

MANAGEMENT

Availability of oral chelators revolutionized the management of WD and changed its dire prognosis to a good outlook. Penicillamine is the amino acid cysteine to which two methyl groups have been attached. Extensive clinical experience attests to the efficacy, and relative safety, of D-penicillamine in children [87,93]. The dosage of D-penicillamine in children is 20 mg/kg/day, divided into two to three equal doses. If possible, D-penicillamine should be taken well away from mealtime. Vitamin B6 supplementation (25–50 mg daily) is customary. Adverse side effects, such as pancytopenia and proteinuria, are a major problem: the drug has to be stopped in ~30% of patients. As with adults, clinical deterioration of hepatic or neurologic disease when D-penicillamine is started has been reported in children [94,95]. Starting D-penicillamine at gradually increasing doses over the first 10–14 days of treatment may minimize the risk of the so-called hypersensitivity syndrome. In the first 2–3 months of treatment with D-penicillamine, very close monitoring is required to identify any adverse reactions immediately. Trientine is structurally very different form D-penicillamine. It is safe and effective for WD patients intolerant of D-penicillamine, including children [91,96,97]. Many in the United States advocate it as the preferred first-line oral chelator for any pediatric WD patient, not merely children predicted to be at risk for adverse effects associated with D-penicillamine. Trientine itself has few adverse side effects. Dosage in children is also 20 mg/kg/day, divided into two to three equal doses. In general, trientine should be taken away from meals as food can interfere with its already limited absorption.

Zinc influences copper homeostasis in the body by altering expression of metallothioneins in gut and possibly in liver. It interferes with copper absorption and increases fecal copper excretion. In general, hepatic copper concentrations do not change. Zinc is regarded as effective and well tolerated in children [3,98,99]. Recent data cast some doubt on this enthusiasm [100]. Lack of very long-term follow-up is problematic. Dosage in children is 25 mg elemental zinc three times daily, or twice daily in 5-year olds [101]; teenagers can take the adult dose (50 mg elemental zinc three times daily); optimal dose for toddlers and preschool-aged children remains undetermined. While the actual zinc salt is unimportant, zinc acetate and gluconate are well tolerated, and zinc sulfate seems to be the least well tolerated. It has been associated with gastric or duodenal mucosal erosion/ulceration [102]. Zinc should not be taken with food. Zinc may be more effective for neuro-WD than for hepatic-WD, a relevant consideration since hepatic-WD is more common in children. A child whose hepatic status deteriorates on zinc monotherapy requires oral chelation instead. With pediatric WD, establishing a consistent routine of taking zinc may be difficult because it must be taken three times a day and away from meals/snacks. Zinc salts can cause nausea, vomiting, and epigastric pain—further disincentives to taking it. Changing to a different salt or taking the zinc with a little food may solve this problem.

Tetrathiomolybdate (TTM) is a strong anticopper drug which works by chelation and also by interfering with copper absorption. It is an attractive option for treatment of neuro-WD. A new formulation of tetrathiomolybdate as the bis-choline salt is in development, but it is not yet available for treatment in children.

MEDICAL MANAGEMENT OF CLINICALLY EVIDENT WILSON DISEASE

Most children with WD who have clinical evidence of hepatic or neurologic damage require treatment with an oral chelator. Primary treatment with zinc is often reserved for those who have prominent neurologic involvement. Imaging of the central nervous system, preferably by MRI, should be performed in any child with neurologic WD prior to commencing treatment.

For very severe clinical presentations, mainly decompensated cirrhosis, an induction regimen consists of an oral chelator (D-penicillamine or trientine) and zinc given on a temporally dispersed regimen where some drug is given every 5–6 hours, chelator alternating with zinc. This regimen provides a full dose of chelator plus an adequate dose of zinc in each 24-hour period. It is unwieldy for long-term treatment: after 2–3 months, patients who respond should be transitioned to monotherapy. This regimen appears effective [103–105]. For the patient with decompensated cirrhosis, backup liver transplantation must be arranged because medical treatment failure may occur.

Zinc acetate was approved in the United States as maintenance therapy for WD in 1997. Pediatric patients may be suitable for conversion from oral chelator to zinc, usually after 1–5 years of oral chelation. The patient should be clinically stable with normal liver chemistries and biochemical evidence of good response to chelator therapy. The switch can be immediate (no tapering or ramping up), but close monitoring is required during conversion [83]. Alternatively, pediatric patients have been treated initially with chelation (trientine) for 4–8 months, followed by gradual conversion to zinc including a brief period of combined therapy [96].

MEDICAL MANAGEMENT OF SILENT WILSON DISEASE, INCLUDING THE VERY YOUNG CHILD WITH SILENT WILSON DISEASE

Since many children have silent WD, finding the best treatment for them is important. It is not a "one-size-fits-all" situation. Several biological considerations attain: actual mutation(s), evidence of liver damage, and age. Economic or social considerations may force a treatment decision independent of pathobiology.

Current treatment strategies focus on toxic copper overload. Oral chelators are regarded as aggressive treatment: the aim is to "de-copper" the liver. Zinc is considered gentler, but it is well established that zinc does not radically alter hepatic copper concentration. Extended experience in adults, while limited [106], suggests that zinc may not be effective for hepatic-WD long-term. Overall, pharmacological management of WD requires monitoring and flexibility in drug treatment.

Any child with silent WD who has mutation(s) resulting in failure to produce the Wilson ATPase protein altogether requires aggressive treatment and close monitoring. These individuals likely approximate to the rapidly progressive deterioration of the knockout mouse. Likewise, a child with silent WD and, additionally, silent cirrhosis probably needs oral chelation since evidence for histological improvement in children is based on chelation [107]. Silent WD with abnormal liver biochemistries may respond adequately to zinc or may be recalcitrant. Accruing pediatric experience suggests that close monitoring is mandatory [100]. The patient's age poses various problems. It cannot be assumed that teenagers necessarily have greater hepatic copper concentrations than toddlers. Dosing may be problematic in young children. Adherence may be a game-changer in adolescents.

Specifically for very young children (<5 years-old) with definite WD, best treatment remains undetermined. Although zinc is currently preferred for treatment of very young asymptomatic children, the optimal dose has not been established. The commonsense determination is 25 mg elemental zinc once (toddlers) or twice daily. Early chelation therapy to reduce the hepatic parenchymal copper concentration deserves serious consideration. Any treatment regimen in the very young child must be assessed for risks associated with copper depletion or zinc surfeit in a critical period of growth. For example, if WD is diagnosed in the neonatal period, immediate restriction of dietary copper may interfere with neurologic development. A regular baby formula may thus be appropriate. Delaying treatment to the second year of life may be reasonable if the very young child has no evidence of hepatic or neurologic disorder. Dosing becomes easy once the child weighs 10–12 kg.

In the future, there may be a second therapeutic focus, namely rehabilitation of the defective Wilson ATPase. Decoppering would then take place through the normal function of the rehabilitated protein. This strategy may apply to only selected mutations, for example, H1069Q, but it will enhance the need for genotypic diagnosis.

DIETARY CONSIDERATIONS FOR CHILDREN WITH WILSON DISEASE

WD cannot be treated successfully under any circumstances by dietary manipulation alone. Opinion is divided about the importance of excluding dietary copper in pediatric patients on pharmacological treatment [108]. Some restriction in the first year of treatment may be appropriate for children with abnormal liver tests or clinical illness. The problem is that dietary limitations reinforce the stigma of being unwell. In any case, if a child's dietary copper is to be limited, guidelines must be extremely simple. Patients should avoid liver and organ meats, shellfish, mushrooms, nuts, and chocolate. Vegetarian and vegan patients do require supervision by a dietitian, possibly an issue if an adolescent adopts such dietary practices after achieving clinical stability. If drinking water is suspected to be high in copper, it should be checked: filtered water can be substituted, if necessary. No systematic data are available in children regarding the merits of supplementation with dietary antioxidants such as vitamin E. Other complementary/alternative medicines and dietary supplements [109] may be high in copper.

FOLLOW-UP STRATEGIES FOR THE PEDIATRIC PATIENT ON TREATMENT

Follow-up is similar to that for adults (see Chapter 16: Wilson Disease in Adults: Clinical Presentations, Diagnosis, and Medical Management), with at least twice yearly clinical review. When starting therapy, patients should be advised to report any adverse events; they should have complete blood count, liver and renal biochemistries performed at each review. Pediatric patients maintained on zinc need to be followed specifically for signs of advancing hepatic disease (elevated aminotransferases, thrombocytopenia, ultrasonographic abnormalities). Measuring 24-hour urinary copper excretion is useful in monitoring therapy; measuring 24-hour urinary zinc is useful for monitoring adherence to zinc intake. On oral chelators, urinary copper <200 μg/24 hours (<3 μmol/24 hours) suggests non-adherence or overtreatment. Estimate of the non-ceruloplasmin-bound copper may distinguish between these situations, as it is subnormal (<5 μg/dL; <50 μg/L) in overtreatment and elevated (>15 μg/dL; >150 μg/L) with inadequate treatment or non-adherence [83]. Issues relating to how non-ceruloplasmin-bound copper is estimated limit the utility of this approach (see Chapter 22: Direct Determination of Non-Ceruloplasmin-Bound Copper in Plasma). If the non-ceruloplasmin-bound copper is consistently 0–5 μg/dL (0–50 μg/L), dose of chelator should be gradually reduced and patient monitored. Serial ophthalmologic examinations may be helpful in documenting that the Kayser–Fleischer rings have resolved and vanished, versus development of Kayser–Fleischer rings in those with poor adherence.

CLASSIC WILSONIAN ACUTE LIVER FAILURE AND LIVER TRANSPLANTATION

The child with acute liver failure in WD may benefit from specific interventions as a bridge to transplant, in addition to meticulous supportive care. Specifically, albumin dialysis [110] and related techniques [111,112] may stabilize the pediatric patient. In an adolescent, this type of intervention has on occasion obviated the need for transplant [113]; however, this outcome is exceptional with classic Wilsonian acute liver failure.

Prognostic scoring systems can assist in determining who needs a liver transplant [87]. Liver transplantation can be lifesaving in classic Wilsonian acute liver failure and true treatment failures. For the patient with decompensated cirrhosis, backup liver transplantation should be arranged because medical treatment failure may occur. Living-related donors who are obligate heterozygote carriers are acceptable as donors [114]. Review of the United Network for Organ Sharing database in the United States has shown that those transplanted for WD-related chronic liver disease have higher patient survival rates than those with classic Wilsonian acute liver failure and that children had better survival rates than adults undergoing transplantation for WD [115]. Overall, outcomes for liver transplantation in WD are excellent and comparable to transplantation for other indications (see Chapter 38: Liver Transplantation in Wilson Disease).

CONCLUSION

With improved methods of diagnosis, WD has become an important disease in childhood. The hepatic manifestations are varied, and the neuropsychiatric manifestations may be subtle or nonspecific. Treatment needs to be individualized; comprehensive follow-up is important for all patients. Scoring systems may assist with diagnosis or clinical decisions relating to need for liver transplantation. A recently published position paper complements the views expressed in this chapter [116].

SUMMARY

WD can affect children of all ages. In the current era of increased availability of genetic testing, WD is being diagnosed earlier in life. Indeed, there are over 50 cases of WD in children under 5 years of age documented in the literature. The need to be aware of the possibility of WD as the cause of liver dysfunction regardless of age is important. Clinical presentation may be chronic liver disease, acute liver failure with distinctive features (ALF-WD) or it may be "silent" liver disease. A broad spectrum of neurologic or psychiatric conditions can occur in childhood or adolescence. While hepatic presentation is more common than neurologic in the pediatric population, manifestations of WD are characteristically multisystemic. WD can resemble autoimmune liver disease clinically. Particularly when there is inadequate response to immunosuppression therapy in autoimmune hepatitis, every effort must be made to ensure that a diagnosis of WD is not being missed. A combination of biochemical tests including serum ceruloplasmin, 24-hour urinary copper excretion, and liver copper concentration as well as genetic tests is used to establish diagnosis. Scoring systems to assist diagnosis and to assess prognosis are available. Current management includes chelators like D-penicillamine or trientine; zinc has multiple effects exclusive of chelation. Economic and social factors often dictate drug therapy. Foods containing high

amounts of copper like shellfish and chocolates may be restricted, at least in the first year of treatment. After a diagnosis of WD, first-degree relatives of the child should be screened. Therapy is lifelong. Adherence to medical recommendations is necessary for good outcomes. Parents should be made aware that stopping therapy suddenly may prove fatal for the child. Liver transplantation is required in classic Wilsonian acute liver failure and when medical therapy fails. Newer therapies are being investigated. Newborn screening may become routine in the future.

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Chapter 3

The genetics of Wilson disease

IRENE J. CHANG[1] AND SI HOUN HAHN[2]*

[1]*Division of Medical Genetics, Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA*

[2]*Division of Genetic Medicine, Department of Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle, WA, USA*

Abstract

Wilson disease (WD) is an autosomal-recessive disorder of hepatocellular copper deposition caused by pathogenic variants in the copper-transporting gene, *ATP7B*. Early detection and treatment are critical to prevent lifelong neuropsychiatric, hepatic, and systemic disabilities. Due to the marked heterogeneity in age of onset and clinical presentation, the diagnosis of Wilson disease remains challenging to physicians today. Direct sequencing of the *ATP7B* gene is the most sensitive and widely used confirmatory testing method, and concurrent biochemical testing improves diagnostic accuracy. More than 600 pathogenic variants in *ATP7B* have been identified, with single-nucleotide missense and nonsense mutations being the most common, followed by insertions/deletions, and, rarely, splice site mutations. The prevalence of Wilson disease varies by geographic region, with higher frequency of certain mutations occurring in specific ethnic groups. Wilson disease has poor genotype–phenotype correlation, although a few possible modifiers have been proposed. Improving molecular genetic studies continue to advance our understanding of the pathogenesis, diagnosis, and screening for Wilson disease.

INTRODUCTION

In this chapter, we will discuss the inheritance, gene frequency, variants, genotype–phenotype correlation, and modifiers of the *ATP7B* gene, and the clinical molecular diagnosis and population screening for Wilson disease.

INHERITANCE

Wilson disease is a monogenic autosomal-recessive condition and carriers do not manifest any symptoms. Autosomal-recessive conditions are not usually present in consecutive generations, but may occur in populations with particularly high carrier frequency of Wilson disease (Wu et al., 2015). Our group and others have reported the presence of Wilson disease in two or more successive generations within the same family, reflecting a "pseudo-dominant" inheritance (Dziezyc et al., 2011, 2014; Bennett et al., 2013; H. Park et al., 2015). Therefore, the diagnosis of Wilson disease should not be excluded simply due to a misleading family history consistent with an autosomal-dominant inheritance pattern. Furthermore, recent studies have also identified Wilson disease due to atypical forms of inheritance, such as the presence of three concurrent mutations in a single patient or segmental uniparental disomy (Coffey et al., 2013). Uniparental disomy occurs when both homologs of a chromosome originate from a single parent. These findings have implications for clinical practice and genetic counseling, as clinicians may need to consider genotyping asymptomatic parents or obtaining full sequencing of *ATP7B* to confirm that pathogenic variants occur in *trans*.

ATP7B GENE AND ATPASE

Wilson disease is caused by homozygous or compound heterozygous mutations in the *ATP7B* gene (OMIM# 606882), which encodes a transmembrane copper-transporting P-type ATPase of the same name. Currently,

*Correspondence to: Si Houn Hahn, MD, PhD, Department of Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle WA 98105, USA. Tel: +1-206-987-7610, Fax: +1-206-987-5329, E-mail: sihahn@uw.edu

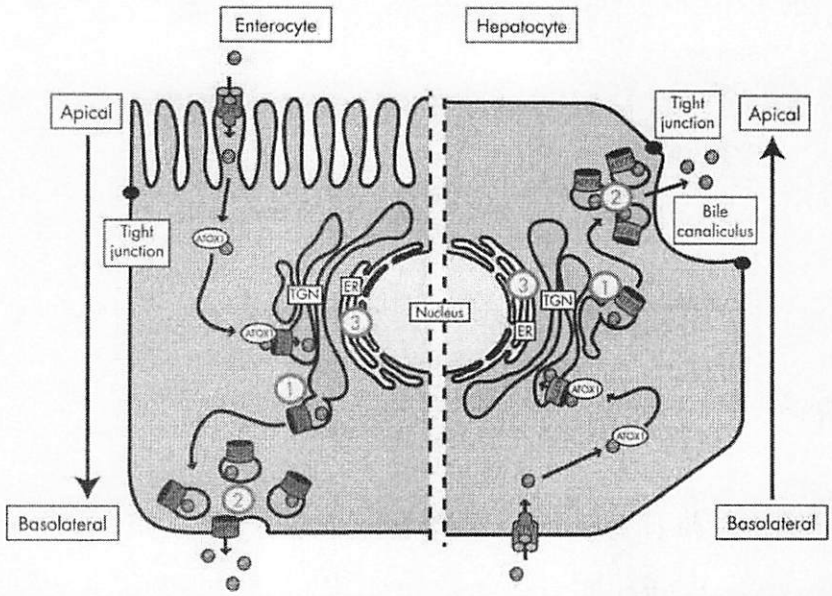


Fig. 3.1. Schematic representation of copper-induced relocalization of ATP7A and ATP7B. The left side of the diagram represents an enterocyte and the right side represents a hepatocyte. On both sides, copper enters the cell through copper transporter 1 (CTR1) and is escorted by copper chaperone antioxidant protein 1 (ATOX1) to ATP7A or ATP7B in the trans-Golgi network (TGN). When copper levels rise above a certain threshold, ATP7A and ATP7B excrete copper into the plasma on the basolateral side of the enterocyte and into the bile on the apical side of the hepatocyte. Defects in localization of ATP7B may lead to copper accumulation at the (1) TGN due to unresponsiveness, (2) cell periphery, and (3) endoplasmic reticulum (ER) due to misfolding. (Reproduced from de Bie et al., 2007.)

ATP7B is the only identified gene known to cause Wil son disease (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). Mutations in the *ATP7B* gene have been reported in almost all exons. Previous studies have reported individuals with both biochemical and clinical diagnosis of Wilson disease in the absence of two *ATP7B* mutations, raising the possibility of a second causative gene (Lovicu et al., 2006; Kenney and Cox, 2007; S. Park et al., 2007; Mak and Lam, 2008; Nicastro et al., 2010; Coffey et al., 2013). Nonetheless, *ATP7B* remains the only known gene responsible for Wilson disease.

Human dietary intake of copper is about 1.5–2.5 mg/day, which is absorbed in the stomach and duodenum, bound to circulating albumin, and transported to the liver for regulation and excretion (Culotta and Scott, 2016). The uptake of copper occurs on the basolateral side of hepatocytes via copper transporter 1 (CTR1), as illustrated in Figure 3.1. A specific copper chaperone, antioxidant protein 1 (ATOX1), delivers copper to the Wilson disease protein, ATP7B, by copper-dependent protein–protein interactions (Walker et al., 2004). Within hepatocytes, ATP7B performs two important functions in either the trans-Golgi network (TGN) or in cytoplasmic vesicles. In the TGN, ATP7B activates ceruloplasmin by packaging six copper molecules into apoceruloplasmin, which is then secreted into the plasma. In the cytoplasm, ATP7B sequesters excess copper into vesicles and excretes it via exocytosis across the apical canalicular membrane into bile (Bull et al., 1993; Tanzi et al., 1993; Yamaguchi et al., 1999; Cater et al., 2007). Due to the binary role of the ATP7B transporter in both the synthesis and excretion of copper, defects in its function lead to copper accumulation and the progressive features of Wilson disease (Fig. 3.1).

MOLECULAR STRUCTURE OF *ATP7B*

ATP7B is located on 13q14.3 and contains 20 introns and 21 exons, for a total genomic length of 80 kb (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). The gene is synthesized in the endoplasmic reticulum, then relocated to the TGN within hepatocytes. *ATP7B* is most highly expressed in the liver, but is also found in the kidney, placenta, mammary glands, brain, and lung.

ATP7B (P-TYPE ATPASE) PROTEIN STRUCTURE AND FUNCTION

ATP7B belongs to class 1B (PIB) of the highly conserved P-type ATPase superfamily, which is responsible for the transport of copper and other heavy metals across cellular membranes (Gourdon et al., 2011). The protein contains 1465 amino acids, a phosphatase domain (A-domain), phosphorylation domain (P-domain, amino acid residues 971–1035), nucleotide-binding domain (N-domain, amino acid residues 1240–1291), and

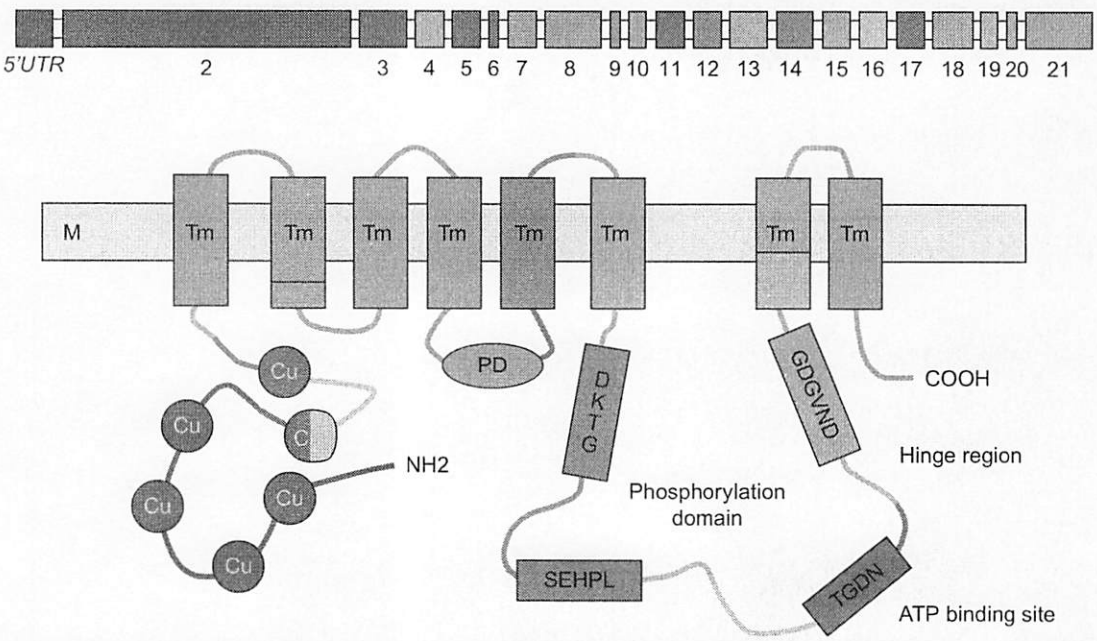


Fig. 3.2. Schematic representation of *ATP7B* gene and corresponding human ATP7B protein. Top diagram shows 5'UTR promoter region and exons separated by introns. Bottom diagram shows the domain organization of human copper ATPase. Conserved amino acid motifs are present at the core structure of each functional domain, i.e., TGDN and GDGVND at the A-domain, DKTG at the P-domain, and SEHPL in the N-domain. M, phospholipidic bilayer of the membrane; Cu, the metal-binding domains of the trasmembrane cation channel; Tm, transmembrane domains; PD, phosphatase domain. (Reproduced from Fanni et al., 2005.)

M-domain, which comprises eight transmembrane ion channels (Fig. 3.2) (Cater et al., 2004, 2007; Lenartowicz and Krzeptowski, 2010).

Unique amino acid motifs are present at the core structure of each domain, such as TGEA at the A-domain, DKTGT at the P-domain, and SEHPL in the N-domain. Specifically, the N-terminal metal-binding domain (MBD) is composed of six copper-binding sites, each with the conserved sequence motif GMXCXXC (Fatemi and Sarkar, 2002; Sazinsky et al., 2006). These MBDs play a central role in accepting copper from copper chaperone ATOX1 through protein–protein interactions. Previous studies have demonstrated unequal impact of MBDs on ATP7B activity, with MBD 5 and 6 having stronger effects on the catalytic activation of ATP7B than MBDs 1–4 (Lutsenko et al.,1997).

The active transport of copper across membranes is a complex process that begins with ATP7B binding copper at the N-terminal domain and transporting it across cellular membranes, using ATP as an energy source (Fig. 3.2). Next, free copper binds intracellularly to GG motifs in the MBDs, followed by transport on to the Cys-Pro-Cys (CPC) sequence motifs in MBD 6. Finally, dephosphorylation of acyl-phosphate at the A-domain discharges copper across the cellular membrane. Mutations causing copper accumulation may occur at any of these steps (Huster et al., 2006; Schushan et al., 2012).

Although the mechanism by which the histadine-containing SEHPL motif affects copper transport remains to be elucidated, it is clear that histidine-to-glutamate substitution at amino acid 1069 (p.H1069Q) in this motif is the most common cause of Wilson disease in northern Europeans. In the hepatocytes of patients homozygous for p.H1069Q, ATP7B was found in the endoplasmic reticulum instead of its usual TGN location, suggesting abnormal protein trafficking (Huster et al., 2003). Insect models with the p.H1069Q mutation in SF9 cells showed decreased ATP-mediated catalytic phosphorylation but no major protein misfolding, suggesting a role for p.H1069Q in the orientation of the ATP7B catalytic site for ATP binding prior to hydrolysis (Tsivkovskii et al., 2003).

VARIANTS IN THE *ATP7B* GENE

More than 600 pathogenic variants in *ATP7B* have been identified, with single-nucleotide missense and nonsense mutations being the most common, followed by insertions/deletions and splice site mutations (Human Gene Mutation Database, accessed 29 April 2016; Stenson et al., 2014). Other rare genetic mechanisms that have been reported in the literature include whole-exon deletions, promoter region mutations, three concurrent pathogenic variants, and monogenic disomy (Coffey et al., 2013; Bandmann et al., 2015). Mutation "hotspots" in

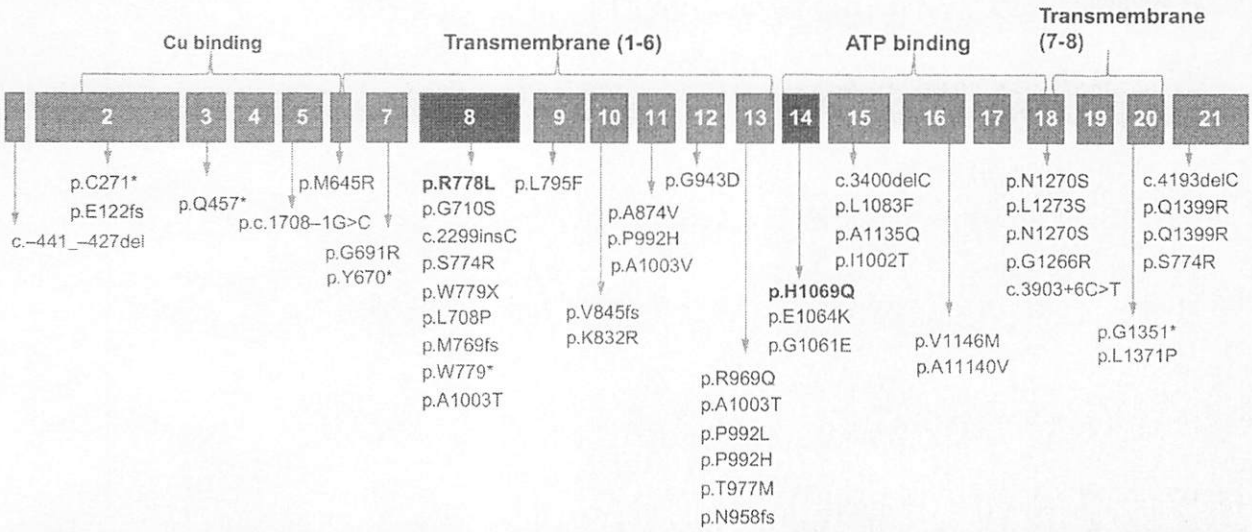


Fig. 3.3. Schematic of the *ATP7B* gene with common mutation sites, including p.H1069Q (rs76151636), p.R778L (rs28942074), p.E1064K (rs376910645), c.3400delC, and p.Ala1135fs (rs137853281). Please refer to Table 3.1 for more details.

ATP7B have also been reported to vary by geographic region (see regional gene frequency section, below). The majority of pathogenic mutations are located in the M- and N-domains in presymptomatic patients or in those with hepatic symptoms (S. Park et al., 2007). The common mutations in *ATP7B* seen in various populations are listed in Figure 3.3.

The p.H1069Q mutation is one of the most common mutations, with a population allelic frequency of 10–40% (30–70% among Caucasians). Most patients are compound heterozygotes, carrying different mutations on each copy of the chromosome (Usta et al., 2014). The p.H1069Q mutation occurs when histidine of the conserved SEHPL motif in the N-domain of *ATP7B* is replaced by glutamic acid, resulting in N-domain protein misfolding, abnormal phosphorylation in the P-domain, and decreased ATP binding affinity (Rodriguez-Granillo et al., 2008). This mutation also leads to decreased heat stability and abnormal localization of the protein to the TGN (Ralle et al., 2010).

Other common mutations in *ATP7B* include p.E1064A, p.R778L, p.G943S, and p.M769V. Mutations in p.E1064A, also found in the SEHPL motif, completely disable ATP binding affinity but do not result in protein misfolding, transport abnormalities, or thermal instability. The p.R778L mutation affects transmembrane transport of copper (Dmitriev et al., 2011). The p.G943S and p.M769V mutations result in defective copper metabolism but preserved ceruloplasmin levels (Okada et al., 2010).

A substantial proportion of Wilson disease-associated missense mutations, including p.H1069Q and p.R778L, result in markedly decreased level of the protein caused by enhanced degradation (Payne et al., 1998; de Bie et al., 2007; van den Berghe et al., 2009). Other prevalent mutations, such as protein-truncating nonsense mutations (~13% of known point mutations) (Merle et al., 2010) and frameshift mutations (Vrabelova et al., 2005), are predicted to cause decay of mRNA (Mendell et al., 2004; Chang et al., 2007) or a severely truncated protein, resulting in absent or diminished levels of protein. It is therefore expected that most patients with Wilson disease have absent or significantly reduced levels of ATP7B.

REGIONAL GENE FREQUENCY

The prevalence of Wilson disease varies by geographic region, with higher prevalence of specific mutations reported in certain populations (Ferenci, 2006) (see Chapter 2 for more details). A list of the common regional variants of ATP7B mutations and geographic clustering of mutations are shown in Table 3.1 and Figure 3.4, respectively.

GENOTYPE–PHENOTYPE CORRELATION

Direct genotype–phenotype relationships in Wilson disease have been difficult to establish, despite several studies examining correlation (Panagiotakaki et al., 2004; Vrabelova et al., 2005; Nicastro et al., 2010; Cocoş et al., 2014; Usta et al., 2014). The numerous low-frequency and compound heterozygous nature of Wilson disease obfuscate the process of characterizing its numerous genetic variants and their clinical consequences. Descriptions of phenotypes are limited to age of onset and presenting symptoms, both of which may be affected by inaccurate diagnostic criteria, delayed diagnosis, and practitioner selection bias. Therefore,

Table 3.1

Regional distribution of common Wilson disease mutations by geographic location

Region	AF (%)	Prevalent mutations					
		Protein	Nucleotide	RS	Exon	Type	Domain
Europe							
Austria (**Ferenci**, 2006)	34.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	6.4	p.Gly710Ser	c.2128G>A		8	Missense	TM2
	3.6	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Benelux (**Ferenci**, 2006)	53	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Bulgaria (**Todorov** et al., 2005)	58.8	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Canary Islands (**García Villarreal** et al., 2000)	64	p.Leu708Pro	c.2123 T>C		8	Missense	TM2
Czech Republic (**Vrabelova** et al., 2005)	57	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Denmark (**Møller** et al., 2011)	18	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	16	p.Trp779*	c.2336G>A	rs137853283	8	Nonsense	TM4
France (**Bost** et al., 2012)	15	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Germany (**Ferenci**, 2006)	47.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Germany (East, former) (**Caca** et al., 2000)	63	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Greece (**Panagiotakaki** et al., 2004; **Dedoussis** et al., 2005; **Gomes** and **Dedoussis**, 2016)	35	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	12	p.Arg969Gln	c.2906G>A	rs774028495	13	Missense	TM6
Hungary (**Firneisz** et al., 2002; **Folhoffer** et al., 2007)	42.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Iceland (**Thomas** et al., 1995a; **Hofer** et al., 2012)	100	p.Tyr670*	c.2007_2013del		7	Nonsense	TM1
Italy (**Loudianos** et al., 1999)	17.5	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	9	p.Val845fs	c.2530delA	rs755709270	10	Premature stop	Td
	6	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Netherlands (**Stapelbroek** et al., 2004)	33	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop

Continued

Table 3.1

Continued

Region	AF (%)	Prevalent mutations		RS	Exon	Type	Domain
		Protein	Nucleotide				
Poland (**Gromadzka** et al., 2005)	72	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	7.3	p.Ala1135fs	c.3400delC	rs137853281	15	Premature stop	ATP loop
	3.7	p.Gln1351*	c.4051C>T		20	Nonsense	
Romania (**Iacob** et al., 2012)	38.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Russia (**Ivanova-Smolenskaya** et al., 1997)	49	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Sardinia (**Figus** et al., 1995)	60.5		c.-441_-427del		5prime	Unknown	Promoter
	8.5	p.Met822fs	c.2463delC		10	Deletion	TM4/Td
	7.9	p.Val1146Met	c.3436G>A		16	Missense	ATP loop
Serbia (**Tomić** et al., 2013)	38.4	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	11.6	p.Met769fs	c.2304dupC		8	Missense	TM4
	9.3	p.Ala1003Thr	c.3007G>A	rs1801247	13	Missense	TM6/Ph
Spain (**Margarit** et al., 2005)	27	p.Met645Arg	c.1934 T>G	rs121907998	6	Missense	Cu6/TM1
Sweden (**Shah** et al., 1997)	38	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Turkey (**Ferenci**, 2006; **Simsek Papur** et al., 2013)	17.4	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	5.3	p.Gly710Ser	c.2128G>A	rs772595172	8	Missense	TM2
	4.53	p.Gln457*	c.1369C>T		3	Nonsense	Cu4/Cu5
UK (**Coffey** et al., 2013)	19	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	8	p.Met769Val	c.2305A>G		8	Missense	TM4
Yugoslavia (former) (**Loudianos** et al., 1999)	48.9	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	11.4	p.Met769fs	c.2298_2299insC	rs137853287	8	Premature stop	TM4
Asia							
China (**Gu** et al., 2003; **Z.-Y. Wu et al.**, 2003; **Wang** et al., 2011; **Wei** et al., 2014)	31	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	10	p.Pro992Leu	c.2975C>T	rs201038679	13	Missense	TM6/Ph
	9.6	p.Ile1148Thr	c.3443 T>C	rs60431989	16	Missense	ATP loop
	3.3	p.Thr935Met	c.2804C>T		12	Missense	TM5
	19	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4

North India (**S. Kumar** et al., 2006; **Gupta** et al., 2007)							
	12	p.Ile1102Thr	c.3305 T>C	rs560952220	15	Missense	ATP loop
	9	p.Pro992His	c.2975C>A		13	Missense	TM6/Ph
South India	11	p.Ala1003Val	c.3008C>T		13	Missense	TM6/Ph
(**Santhosh** et al., 2006; **S. S. Kumar** et al., 2012)							
	11	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3
	9	p.Pro768Leu	c.2303C>T		8	Missense	TM4
	9	p.Arg969Gln	c.2906G>A	rs121907996	13	Missense	TM6
East India	16	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3
(**Gupta** et al., 2005)							
	11	p.Gly1061Glu	c.3182G>A		14	Missense	ATP loop
	8.5		c.1708-1G>C	rs137853280	5	Splice	Cu6
West India	20	p.Cys271*	c.813C>A	rs572147914	2	Nonsense	Cu3
(**Aggarwal** and **Bhatt**, 2013; **Aggarwal** et al., 2013)							
	11	p.Glu122fs	c.365_366delins TTCGAAGC		2	Ins/Del	Cu1
	6	p.Thr977Met	c.2930C>T	rs72552255	13	Missense	TM6
	6	p.Leu795Phe	c.2383C>T		9	Missense	TM4/Td
Japan	17.95	p.Asn958fs	c.2871delC		13	Premature stop	TM5/TM6
(**Okada** et al., 2000; **Tatsumi** et al., 2010)							
	16.7	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	10.5		c.1708-5 T>G		5	Splice	Cu6
Korea	37.9	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
(**E. K. Kim** et al., 1998; **Yoo**, 2002; **G.-H. Kim** et al., 2008; **Song** et al., 2012)							
	12.1	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge
	9.4	p.Ala874Val	c.2621C>T	rs376355660	11	Missense	TM5
	8	p.Leu1083Phe	c.3247C>T		15	Missense	ATP loop
Lebanon	44.7	p. Ala1003Thr	c.2299insC	rs137853287	8	Missense	TM4
(**Usta** et al., 2014)							
Saudi Arabia	32	p.Gln1399Arg	c.4196A>G		21	Missense	After TM8
(**Al Jumah** et al., 2004; **Majumdar** et al., 2004)							
	16	p.Ser774Arg	c.2230 T>C	rs535217574	21	Missense	TM3
Taiwan	29.6	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
(**Lee** et al., 2000; **Wan** et al., 2006)							
	8.9	p.Pro992Leu	c.2975C>T	rs201038679	13	Missense	TM6
	4.8	p.Gly943Asp	c.2828G>A		12	Missense	TM5

Continued

Table 3.1

Continued

Region	AF (%)	Prevalent mutations					
		Protein	Nucleotide	RS	Exon	Type	Domain
Thailand (**Panichareon** et al., 2011)	10.52	p.Arg778Leu	c.2332C>T	rs28942074	8	Missense	TM4
	7.89	p.Leu1371Pro	c.4112 T>C		20	Missense	TM8
Iran (**Zali** et al., 2011)	19	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
Africa							
Egypt (**Abdelghaffar** et al., 2008; **Abdel Ghaffar** et al., 2011)	42.2	IVS18+6 T>C	c.3903+6C>T	rs2282057	18	Splice	
	40.6	p.Ala11140Val	c.3419C>T		16	Missense	ATP loop
	26.5	p.Lys832Arg	c.2495A>G	rs1061472	10	Missense	TM4/Td
Americas							
USA (**Kuppala** et al., 2009)	40.3	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	1.9	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge
	1.9	p.Gly1266Arg	c.3796G>A	rs121907992	18	Missense	ATP hinge
Brazil (**Deguti** et al., 2004; **Machado** et al., 2008; **Bem** et al., 2013)	37.1	p.His1069Gln	c.3207C>A	rs76151636	14	Missense	ATP loop
	31.25	p.Ala1135fs	c.3400delC	rs137853281	15	Premature stop	ATP loop
	11.4	p.Ala1135GlnfsX13	c.3402delC	rs137853281	15	Premature stop	ATP loop
		p.Leu708Pro	c.2123 T>C		8	Missense	TM2
Costa Rica (**Shah** et al., 1997)	61	p.Asn1270Ser	c.3809A>G	rs121907990	18	Missense	ATP hinge
Venezuela (**Paradisi** et al., 2015)	26.9	p.Ala1135GlnfsX13	c.3402delC	rs137853281	15	Premature stop	ATP loop
	9.6	p.Gly691Arg	c.2071G>A		7	Missense	TM2

AF, allelic frequency; RS, Reference single nucleotide polymorphism (SNP) cluster identification number.

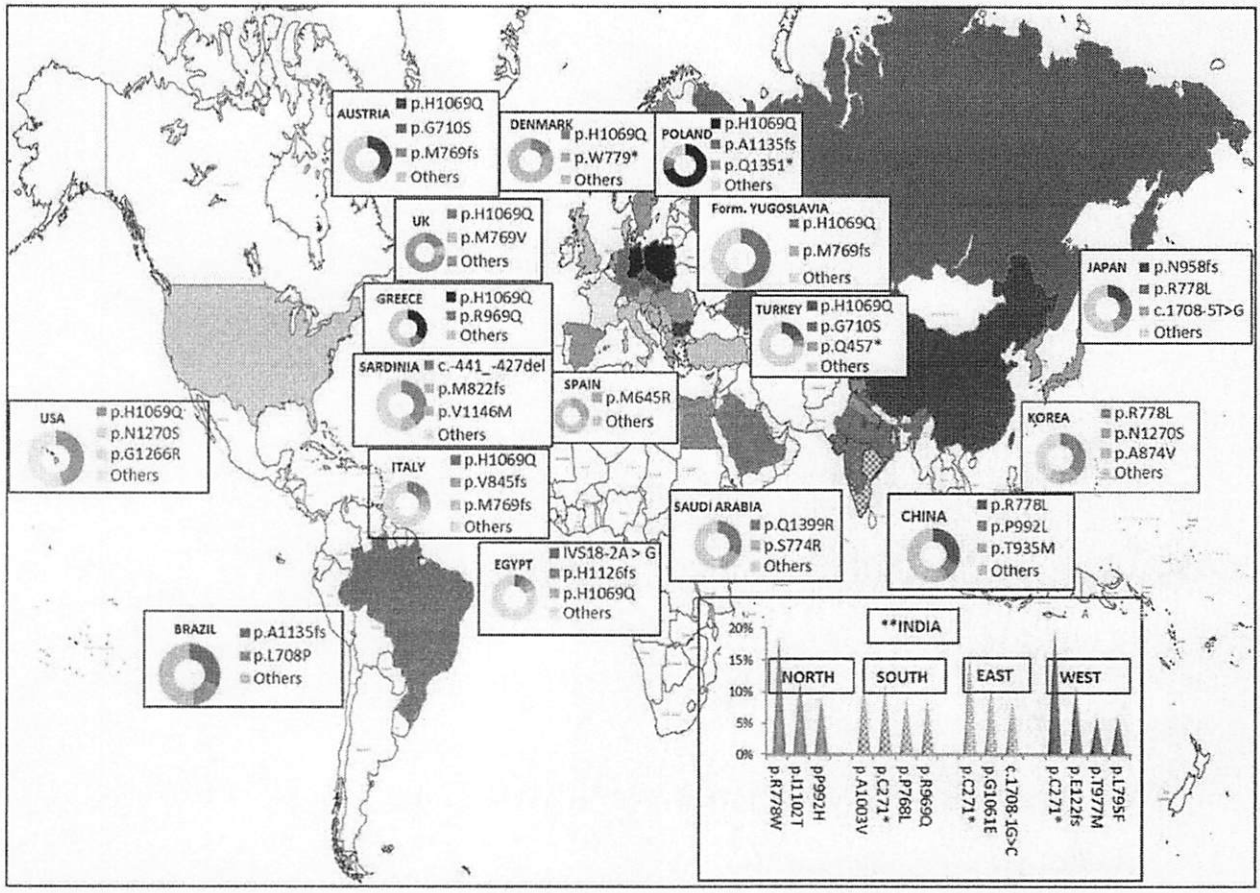


Fig. 3.4. Prevalence of *ATP7B* mutation by geographic region; the darker the gradient, the higher the allelic frequency. (Reproduced from Gomes and Dedoussis, 2016, with permission from Taylor and Francis.)

the marked variability in phenotype of Wilson disease is likely attributable to an amalgamation of genetic, metabolic, and environmental factors (Leggio et al., 2006).

The most consistent genotype–phenotype correlation in Wilson disease is that the most severe, early-onset disease with predominantly hepatic presentation is associated with mutations causing absent ATPase activity. Convincing studies have demonstrated fulminant hepatic disease in mouse models such as the toxic milk (tx) mouse and the Jackson tx mouse (tx^J), which harbor point mutations causing loss of ATP7B function, but not affecting ATP7B synthesis (Theophilos et al., 1996; Coronado et al., 2001; La Fontaine et al., 2001; Huster et al., 2006).

Genetic polymorphisms in *ATP7B*, other genes, and epigenetic factors have been shown to impact disease phenotype by affecting ATP7B protein structure and function. Of the over 600 mutations associated with Wilson disease, the majority are missense mutations that completely inactivate the copper-transporting function of ATP7B (Lutsenko, 2014). In general, individuals with protein-truncating mutations have earlier onset of disease due to decreased protein stability and quantity (Merle et al., 2010). However, other studies have demonstrated partial preservation of copper-transporting function, perhaps explaining the milder phenotypes associated with certain mutations (Rodriguez-Granillo et al., 2008; Dmitriev et al., 2011; Huster et al., 2012). Individuals with the R778L mutation have been shown to have an earlier onset of disease and predominantly hepatic presentation (Z. Y. Wu et al., 2003). In contrast, individuals with the H1069Q mutation have a mean onset of symptoms between 20–22 years old and predominantly neurologic phenotype (Stapelbroek et al., 2004; Kalita et al., 2010). There is also some evidence that Kayser–Fleischer rings are more common in H1069Q homozygous patients in Hungary at time of diagnosis than in compound heterozygous individuals (Folhoffer et al., 2007).

Moreover, pathogenic variants may affect ATP7B targeting from the TGN to cytosolic vesicles. For instance, the p.Met875Val mutation results in a less stable protein and causes reversible ATP7B localization defects. Under a low-copper environment, the p.Gly875Arg variant is sequestered in the endoplasmic reticulum. However, addition of exogenous copper to the cellular growth medium stabilizes the protein, allowing it to complete

its intended journey to the TGN and overcoming its disease-causing phenotype. Theoretically, patients with this specific variant may be more sensitive to dietary copper deficiency (Gupta et al., 2011).

The timing and location of copper buildup can also preferentially alter the hepatic transcriptome, based on homozygous *ATP7B*$^{-/-}$ mouse models. Proteomic analyses of mRNA profiles at each of these disease stages reflect unique patterns (Huster et al., 2006; Ralle et al., 2010). In the initial stage, mRNA for proteins responsible for cell cycle regulation, splicing, and cholesterol synthesis is present (Burkhead et al., 2011). This leads to early accumulation of copper bound to metallothioneins in the cytosol and free copper in the nuclei. In the progressive stage, mRNA changes throughout the cell are present, including the endoplasmic reticulum, mitochondria, and endocytic pathways, causing copper to pathologically accumulate within hepatocytes. In the later stages, mRNA for lysosomal and endosomal proteins is upregulated. In these final stages, copper concentrations decrease in the cytosol and nuclei, and accumulate in the membranous cellular compartment, causing bile duct proliferation and hepatic neoplastic changes. Therefore, the location of copper accumulation may convey more specific prognostic information about disease progression rather than total copper levels.

Other studies have compared homozygotes to compound heterozygotes of the same mutation to establish genotype–phenotype correlations. A study of 76 members of a large, consanguineous Lebanese family showed an association between c.2299insC and hepatic disease and between the p.Ala1003Thr mutation and neurologic disease (Usta et al., 2014).

Other candidate polymorphisms that are thought to modify the clinical phenotype of Wilson disease include MTHFR (Gromadzka et al., 2005), COMMD1 (Weiss, 2006), ATOX1 (Simon, 2008), XIAP (Weiss et al., 2010), PNPLA3 and hepatic steatosis (Stättermayer et al., 2012), and DMT1 (Przybyłkowski et al., 2014), although none of these genes has been demonstrated to have significant diagnostic or predictive value.

Significant phenotypic variation of Wilson disease exists between individuals with the same mutation, individuals within the same family, and even between monozygotic twins (Członkowska et al., 2009; Kegley et al., 2010). While some studies have documented high intrafamilial concordance of clinical symptoms and biochemical results (Hofer et al., 2012; Chabik et al., 2014; Ferenci et al., 2015), others have reported a wide range in age of onset and presenting symptoms amongst siblings (Ala et al., 2007; Taly et al., 2007) and families carrying the same mutation (Takeshita et al., 2002). Indeed, disparate clinical presentations in monozygotic twins raise the suspicion for epigenetic modifiers in Wilson disease. See Chapter 4 for more details about the genetic and environmental modifiers of Wilson disease.

CLINICAL MOLECULAR DIAGNOSIS

The current gold standard of diagnosis for Wilson disease is direct Sanger sequencing of the *ATP7B* gene or molecular testing for previously identified familial mutations. Historically, most pathogenic variants in *ATP7B* were identified using a combination of polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temporal temperature gradient electrophoresis (TTGE), denaturing high-performance liquid chromatography (DHPLC), and Sanger sequencing (Loudianos et al., 1999; Shimizu et al., 1999; Margarit et al., 2005; Vrabelova et al., 2005; G. H. Kim et al., 2008). The critical demerits of this complex tiered approach are that the detection rate is not high enough to find most mutations and the turnaround time is often extended. Although regional clusters of specific mutations have been well described, a customized screening approach taking into account these regional variants may be complicated by ethnically diverse populations and inaccurate clinical information provided with samples. Biochemical results are often imprecise, as elevations in urinary copper excretion tend to occur late in the disease process and fewer than 40% of presymptomatic patients excrete copper less than 100 μg/day (Sternlieb and Scheinberg, 1968; Nakayama et al., 2008). For these reasons, direct sequencing of the *ATP7B* gene has become the preferred standard and provides the greatest yield in clinical molecular diagnosis. Please refer to Chapter 14 for details about the diagnosis of Wilson disease.

Starting the diagnostic process with molecular testing may significantly reduce the need for invasive liver biopsy. Liver copper content alone was found to be insufficient to exclude Wilson disease, as levels may not be elevated in some affected patients. Based on several previous studies, biallelic pathogenic variants were identified in about 80% of patients with biochemical and clinical tests suggestive of Wilson disease. Currently available screening tests may not definitively rule out the disease, and no single test could permit *de novo* diagnosis. Of note, many patients may not possess the characteristic findings and may present when their clinical disease is relatively mild. Inappropriate treatment for false-positive cases has the potential of inducing copper deficiency, which can result in hematologic and neurologic sequelae (N. Kumar et al., 2003). These findings reinforce the need for reliable clinical diagnostic criteria

and underscore the benefits of DNA testing prior to invasive procedures (Ferenci, 2005).

Multiplex PCR is used to amplify all 21 exons and splice sites of *ATP7B*, including promoter regions. Although the large deletions or duplications cannot be detected with this conventional Sanger sequencing method, the chance of these being present in Wilson disease appears low (Stenson et al., 2012). If clinical suspicion is still high with only one pathogenic variant identified, then multiplex ligation-dependent probe amplification (MLPA) test should be considered. Microarray-based comparative genomic hybridization is another option to evaluate partial or full gene deletions or duplications with higher sensitivity. Cases with only one pathogenic variant present should be carefully reviewed in the context of other biochemical and clinical findings. Molecular genetic testing using direct mutation analysis is very effective in identifying affected patients and presymptomatic siblings of probands (Manolaki et al., 2009).

Wilson disease is an autosomal-recessive disorder, which means that there is a 25% chance that a full sibling of the index case is also affected. Once homozygous or compound heterozygous mutations in *ATP7B* have been established in the index patient, mutation detection becomes valuable in family screening. The same genotype in asymptomatic family members confirms diagnosis of the disease, thus allowing for early treatment before the onset of complications. In family members in whom clinical and biochemical features are uncertain, the demonstration of either heterozygous (carrier) or wild-type gene sequence prevents unnecessary treatment (Chang et al., 2007).

If the proband has secured a diagnosis of Wilson disease on the basis of clinical and biochemical evidence, but testing for *ATP7B* mutations is not available, family screening can be done by haplotype analysis of polymorphic markers flanking the disease gene (Thomas et al., 1995b; Gupta et al., 2005; Przybyłkowski et al., 2014). In this instance, the rare possibility of recombination events (typically 0.5–5% of cases) needs to be considered. The rate of recombination is dependent on which flanking markers are studied. Microsatellite or single-nucleotide polymorphisms in the *ATP7B* lateral wing are used for haplotyping, which is useful for screening relatives of patients with previously identified familial mutations. False-positive results may occur if haplotyping is used on patients with low-probability gene recombinations.

Genetic testing for *ATP7B* mutations can be valuable to confirm a diagnosis of Wilson disease, especially when presentation is unusual (Caprai et al., 2006). Attention has been drawn to this situation by the molecular confirmation of early-onset hepatic disease in a 3-year-old child (Wilson et al., 2000). Mutation analysis has also confirmed late-onset disease, including the case of two siblings in their 70s – the oldest reported patients so far at time of diagnosis (Nanji et al., 1997; Gupta et al., 2005; Perri et al., 2005; Weitzman et al., 2014).

ATP7B mutation analysis makes an important contribution to clinical practice. Unfortunately, systematic genetic testing for Wilson disease is still difficult and fairly expensive due to the plethora of different mutations, the occurrence of regulatory mutations in noncoding sequence, the large size of the gene, and the limitations of currently available methods. However, technical advances allowing high-throughput screening could be applied to the disease (Bost et al., 2012; Lepori et al., 2012). This new apparatus can sequence six million basepairs of DNA per hour with accuracy greater than 99%. Such advances might permit specialized laboratories to detect all variants by sequencing the entire genomic Wilson disease gene from patients, including not only the translated exons, but also the important noncoding sequences that are not normally investigated.

Interpretation of variants of uncertain significance has become a major challenge for accurate interpretation, genetic counseling, and prevention. Screening family members may help with the interpretation of variants of uncertain significance, but not all variants can be resolved with this approach. Functional analysis is often necessary; however, no clinical functional analysis is currently available. A computational approach to predict significance of mutations is often helpful, but a further concrete model is required to demonstrate the efficacy in guiding clinical decisions.

POPULATION SCREENING

The purpose of newborn screening is to identify treatable congenital conditions that can affect a child's long-term health and development. Recent tandem mass spectrometry (MS/MS) applications have markedly expanded the ability to screen for >50 metabolic diseases from a single dried blood spot. In addition to the original Wilson–Jungner classic screening criteria (Wilson and Jungner, 1968), the American College of Medical Genetics convened the Newborn Screening Expert Group to develop a uniform screening panel in 2006 (American College of Medical Genetics Newborn Screening Expert Group, 2006). Of the primary tenants, Wilson disease is an ideal target for screening, given its relatively high prevalence and availability of effective treatment (Hahn et al., 2002; Roberts et al., 2008). Unfortunately, despite extensive discussion on the need for population screening, no cost-effective biomarkers or methods for early detection have been developed for Wilson disease yet. Several

small pilot studies have been conducted using ceruloplasmin as a biomarker for screening, with limited findings (Yamaguchi et al., 1999; Hahn et al., 2002; Owada et al., 2002; Schilsky and Shneider, 2002; Kroll et al., 2006). Ceruloplasmin alone is not sufficient to screen for Wilson disease in newborns, as a substantial number of newborns present with physiologically low ceruloplasmin. Ceruloplasmin assay around 3 years of age may be the most appropriate population-screening method, but mandatory health checkups at this age are not universally available in the USA and worldwide.

Many treatable congenital disorders are caused by mutations that result in absent or diminished levels of proteins; thus, protein biomarkers have enormous potential in the diagnosis/screening of congenital disorders. Liquid chromatography mass spectrometry with multiple reaction monitoring (LC-MRM-MS) has emerged as a robust technology that enables highly precise, specific, multiplex quantification of signature proteotypic peptides as stoichiometric surrogates of biomarker proteins.

Our lab is currently exploring the use of peptide immunoaffinity enrichment (Whiteaker et al., 2010, 2011) to quantify ATP7B in dried blood spot (DBS). These promising proof-of-concept data open up the possibility of screening for Wilson disease in newborns. Further clinical validation on a large-scale study will be required to determine the efficacy of the assay.

CONCLUSION

Wilson disease is an autosomal-recessive disease due to pathogenic mutations in *ATP7B*. *ATP7B* is the only identified gene known to cause Wilson disease, and encodes a transmembrane copper-transporting ATPase of the same name. While biochemical testing and clinical criteria may assist in the early diagnosis and treatment, the current gold standard for Wilson disease diagnosis is direct Sanger sequencing of ATP7B or molecular testing for known familial mutations. Genotype–phenotype correlations have been studied extensively but direct causations remain nebulous. Modifier genes may affect the penetrance and phenotypes but a large-scale study for clinical validation is warranted. The overall worldwide prevalence of Wilson disease is 1 in 30 000 individuals, with significant geographic variation. The most common mutation in Northern America and Europe is the missense mutation p.H1069Q and the most common mutation in East Asian populations is the missense p.R778L. Ceruloplasmin alone is insufficient to screen for Wilson disease in newborns. While peptide immunoaffinity assays show promise for newborn screening, further large-scale clinical studies are required to determine efficacy of these population-based screening methods for Wilson's disease.

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Quantification of ATP7B Protein in Dried Blood Spots by Peptide Immuno-SRM as a Potential Screen for Wilson's Disease

Sunhee Jung,[†] Jeffrey R. Whiteaker,[‡] Lei Zhao,[‡] Han-Wook Yoo,[§] Amanda G. Paulovich,[‡] and Si Houn Hahn*[,†,∥]

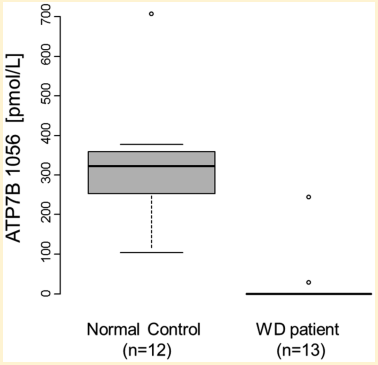
[†]Seattle Children's Hospital Research Institute, Seattle, Washington 98101, United States

[‡]Fred Hutchison Cancer Research Center, Seattle, Washington 98109, United States

[§]Asan Medical Center, Ulsan University College of Medicine, Seoul 05505, South Korea

[∥]Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington 98195, United States

ABSTRACT: Wilson's Disease (WD), a copper transport disorder caused by a genetic defect in the *ATP7B* gene, has been a long time strong candidate for newborn screening (NBS), since early interventions can give better results by preventing irreversible neurological disability or liver cirrhosis. Several previous pilot studies measuring ceruloplasmin (CP) in infants or children showed that this marker alone was insufficient to meet the universal screening for WD. WD results from mutations that cause absent or markedly diminished levels of ATP7B. Therefore, ATP7B could serve as a marker for the screening of WD, if the protein can be detected from dried blood spots (DBS). This study demonstrates that the immuno-SRM platform can quantify ATP7B in DBS in the picomolar range, and that the assay readily distinguishes affected cases from normal controls ($p < 0.0001$). The assay precision was <10% CV, and the protein was stable for a week in DBS at room temperature. These promising proof-of-concept data open up the possibility of screening WD in newborns and the potential for a multiplexed assay for screening a variety of congenital disorders using proteins as biomarkers in DBS.



KEYWORDS: *Wilson's disease, WD, newborn screening, NBS, ATP7B, dried blood spots, DBS, immuno-SRM, peptide immunoaffinity enrichment, mass spectrometry*

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INTRODUCTION

Wilson's Disease (WD) is an autosomal recessive disorder caused by mutations in *ATP7B* gene *(OMIM *606882)*.[1−3] *ATP7B* encodes a transmembrane protein ATPase (ATP7B), which is highly expressed in the liver and kidney and functions as a copper-dependent P-type ATPase. *ATP7B* is required for transmembrane transport of copper from hepatocytes into the biliary system. Absent or reduced function of ATP7B protein results in copper accumulation in the liver and subsequently in the brain, kidneys, and other organs. The impaired function of ATP7B protein also fails to incorporate copper into apoceruloplasmin, resulting in the decreased blood level of ceruloplasmin (CP) in the majority of patients with WD.[4−6]

The prevalence of WD is ∼1 in 30 000 newborns, with a carrier frequency of 1 in 90 (higher in certain populations).[7] However, regional variations exist. In particular, Costa Rica, Sardinia, the Canary Island, and Crete have all reported to have increased incidence.[8−11] WD is a slow, progressive disease. Although the biochemical defects are present from birth, patients with WD typically present with chronic hepatitis, cirrhosis, or acute liver failure in the first or second decade of life. They may have tremors, ataxia, dysarthria, and difficulty swallowing. WD was fatal until treatments were developed a half century ago. In 1955, the identification of D-penicillamine by John Walshe dramatically improved the outcome of WD by increasing the urinary excretion of copper.[12] Other treatments have since been introduced, including trientine and zinc salts, and have proven efficacious.[13−17] Unfortunately, despite the fact that effective medical treatments have been available for over 50 years and can prevent a fatal outcome if patients are diagnosed early, clinically recognizing WD remains difficult because of its slow progression and the broad clinical spectrum of symptoms. Therefore, many patients still present with irreversible multiorgan damage at the time of diagnosis.

Ideally, patients should be recognized in the presymptomatic stage. There has been a consensus that the best way to achieve early diagnosis of WD before the onset of serious symptoms is through NBS.[18−20] The current gold standard for diagnosis of WD includes multiple laboratory tests, such as copper determination in the urine and liver tissue, followed by confirmation with genetic testing of the *ATP7B* gene. These current diagnostic tools, however, are not suitable for large-scale screening.

With the discovery that CP was reduced in the majority (∼85%) of patients with WD,[5,6,20] several methods were developed to measure CP, a proposed marker for WD, in DBS or urine using different analytical platforms such as a sandwich

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ELISA assay and an LC−MS/MS assay.[19,21−25] Unfortunately, from the few pilot studies published measuring CP in infants or children, CP determination alone was insufficient to screen for WD because of high false discovery rates resulting from the fact that (1) CP is physiologically low in some unaffected newborn babies and (2) some heterozygote carriers for WD have reduced levels of CP. Pediatric screening around 3 years of age has been proposed in Japan, but it is practically difficult to screen the entire population.[26] Despite a strong need for more reliable markers or methods to meet the requirement for the universal screening of WD, no further developments have been made in attempting to screen for WD in recent years.

Most WD mutations have been shown to disrupt ATP7B stability, resulting in absent or diminished levels of the protein; thus quantifying ATP7B levels has enormous potential in the screening of WD. There are >370 mutations reported worldwide;[27,28] most are rare and infrequent, except the two most common mutations, p.H1069Q (~37−63% of the white population) and p.R778L (57% of the East Asian population).[8,29−31] In line with previous observations for disease-causing missense mutations,[32−35] some WD-associated missense mutations including p.H1069Q and p.R778L resulted in a markedly decreased level of the ATP7B protein caused by enhanced degradation.[31,36,37] Other prevalent mutations such as protein-truncating nonsense mutations (~13% of known point mutations)[38] and frameshift mutations[30] are predicted to result in the absence or decay of mRNA[39,40] or a severely truncated protein, resulting in absent or diminished levels of the protein. Taken together, it is expected that most patients with WD would have absence of or reduced levels of ATP7B. While these findings suggest that absent or diminished ATP7B levels can be an indicator for WD, it has not yet been tested.

Targeted mass spectrometry, in particular, multiple/selected reaction monitoring (M/SRM), has been used for rapid development of quantitative assays with high specificity, high-throughput, precision, and robustness,[41−45] and cross-laboratory (including international) transferability of SRM-based assays has been achieved.[45] The combination of DBS with SRM is the standard analytical approach in clinical or NBS laboratories for a variety of small metabolites that accumulate as a result of inborn errors of metabolism.[46,47] Although SRM is capable of quantifying proteins present in μg/mL and higher concentrations, many potential protein markers of greatest interest are often in the low ng/mL range. Quantification of proteins and peptides in complex samples (e.g., plasma) in the ng/mL range by SRM is challenging because of the complexity and large dynamic range of the matrix. The sensitivity of SRM is not sufficient to measure low abundance protein markers directly from DBS without an enrichment process due to interferences from more abundant analytes present in the matrix.

In recent years, peptide immunoaffinity enrichment coupled to SRM (immuno-SRM) has emerged as a promising technique for the quantification of low abundance proteins in complex matrices.[48−57] The benefits of immunoaffinity enrichment of the target peptide analyte from digests of complex samples are to greatly enrich the peptide of interest before LC−MS/MS, reducing ion suppression from background components and greatly enhancing the sensitivity of the method. Immuno-SRM has been successfully implemented to address the detection-limit challenges associated with measuring low-abundance protein biomarkers in the low- and sub-μg/L range in a wide array of studies.[48−51,58−62]

While the immuno-SRM technology has been demonstrated on clinical samples,[63,64] it has not yet been adapted for measuring low-abundance proteins from dried blood spots on filter paper. In this study, we proposed to evaluate ATP7B as a marker for WD. As proof-of-concept, we investigated the immuno-SRM methodology and applied this assay to determine the concentrations of ATP7B in DBS from unaffected and WD-affected individuals. The results demonstrate that immuno-SRM is a high-sensitivity platform for DBS analysis of proteins in the low-picomolar range and ATP7B is a potential marker for screening WD in newborns.

EXPERIMENTAL PROCEDURES

Study Approval

Blood samples involved in this study included a total of 13 WD patients and 12 healthy volunteers. Of these, 10 WD and 12 healthy subjects were from Seattle Children's Hospital and 3 WD subjects were from Asan Medical Center in Seoul, Korea. All patients signed informed consent and all research procedures were approved by the institutional review boards of aforementioned institutions. Whole blood from each subject was collected in ACD (acid citrate dextrose) tubes. Dried blood spots were prepared by pipetting 70 μL of blood/spot onto filter paper card (Protein Saver 903 Card, Whatman, Piscataway, NJ), allowed to dry at room temperature overnight, and then stored in sealed plastic bags at −80 °C until use. Fifteen year old DBS samples from proven carriers and affected patient from a previous study[21] were retrieved from −20 °C and tested as described.

Materials

ProteaseMAX Surfactant (no. V2072) was purchased from Promega (Madison, WI). Proteomics grade trypsin, bovine serum albumin protein standard (200 mg/mL), (3-[3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (CHAPS, no. PI28300) detergent, and ammonium bicarbonate (XX) were obtained from Sigma Life Science (St. Louis, MO). Acetonitrile (no. A955) and water (no. W6, LCMS optima grade), formic acid (no. PI28905), and phosphate-buffered saline (PBS, no. 10010-023) were obtained from Thermo Fisher Scientific (Waltham, MA). HepG2 cell line was obtained from the ATCC (Manassas, VA).

Generation of Immuno-SRM Assay Reagents

Rabbit polyclonal antibodies were produced by Pacific Immunology (Ramona, CA). Polyclonal antibodies were affinity purified from 25 mL of antiserum. Purified (>95% by HPLC) heavy stable isotope-labeled peptides were obtained from Anaspec (Fremont, CA). For stable isotope-labeled peptides, the C-terminal arginine or lysine was labeled with [^{13}C and ^{15}N] labeled atoms, resulting in a mass shift of +8 or +10 Da, respectively. Aliquots were stored in 5% acetonitrile/0.1% formic acid at −20 °C until use. The antibody was coupled and immobilized to 2.8 μm Protein G magnetic beads (no. 10004D, Invitrogen, Carlsbad, CA) in a 1 μg antibody-to-2.5 μL of beads ratio. In brief, 250 μL of the beads was added to 1.6 mL Eppendorf tubes and washed once with 250 μL of 1× PBS, followed by the addition of 100 μg of antibody and 1× PBS + 0.03% CHAPS (no. 28300, Thermo Scientific) to yield a total 250 μL of volume. The antibodies were allowed to couple to the beads overnight with tumbling at 4 °C. The next day, the antibodies were immobilized onto the beads as follows (the work was performed in a fume hood). The supernatant was

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Table 1. List of Candidate Peptides

peptide	sequence	molecular weight	parent ion	daughter ions[a]
ATP7B 301−313	YDPSCTSPVALQR	1435.7	718.8	579.8 (y11 + 2), 683.4 (y6), 871.5 (y8), 974.5 (y9)
ATP7B 325−339	VSLPDGAEGSGTDHR	1496.7	499.9	599.8 (y12 + 2), 656.30 (y13 + 2), 729.33 (y7), 858.37 (y8)
ATP7B 887−901	ATHVGNDTTLAQIVK	1566.8	523.3	558.4 (y5), 671.4 (y6), 772.5 (y7), 897.4 (b9)
ATP7B 1056−1077	VLAVVGTAEASSE HPLGVAVTK	2134.2	712.4	827.4 (y17 + 2), 876.9 (y18 + 2), 926.5 (y19 + 2), 966.0 (y20 + 2)

[a]Ion type for daughter ions is in parentheses.

discarded, and 250 μL of freshly prepared 20 mM DMP (dimethyl pimelimidate dihydrochloride, no. D8388, Sigma) in 200 mM triethanolamine, pH 8.5 (no. T1377, Sigma) was added. The samples were tumbled for 30 min at room temperature, and the DMP in triethanolamine was discarded. To quench the reaction, 250 μL of 150 mM monoethanolamine (no. 411000, Sigma) was added and the beads were tumbled at room temperature for 30 min. The antibody beads were washed twice using 250 μL of 5% acetic acid + 0.03% CHAPS (5 min of tumbling at room temperate each time), and washed once more using 250 μL of 1× PBS + 0.03% CHAPS. The antibody-beads suspension was finally resuspended in 250 μL of 1× PBS and stored at 4 °C until use.

Trypsin Digestion

From each DBS sample, twenty 3 mm punches (containing ∼3.7 μL of blood per disc) were obtained with a standard leather punch and placed into a 1.7 mL microcentrifuge, followed by the addition of 500 μL of 0.1% ProteaseMax in 50 mM ammonium biocarbonate (pH 8). Tubes were vortex-mixed for 1 h on Eppendorf MixMate (Eppendorf, Hamburg, Germany). At this point, aliquots were reserved for a Bradford assay. Disulfide bond reduction and trypsin digestion were performed in a single step with 2 M DTT and acetonitrile added to final concentrations of 5 mM and 15%, respectively. Trypsin was used in a 1:50 enzyme to protein ratio (w/w). The mixture was incubated in a 37 °C water bath overnight. After a 10 min centrifugation, the supernatant was transferred to a new tube, dried under a nitrogen stream, and stored at −80 °C until use.

Peptide Immunoaffinity Enrichment and Liquid Chromatography−Mass Spectrometry

The DBS digests were resuspended in 1× PBS + 0.03% CHAPS to yield a 1 μg/μL nominal protein digest concentration. Next, ∼2 mg of protein digest was combined with 4.8 μg of the antibody (immobilized on beads) in each tube and tubes were incubated overnight at 4 °C with tumbling. (The total capture volume was 500 μL.) The beads with immobilized antibodies and captured peptides were washed twice in PBS buffer + 0.03% CHAPS and washed once in PBS diluted 1:10, and peptides were eluted in 30 μL of 5% acetic acid/3% acetonitrile. The elution was frozen at −80 °C until analysis. An Eksigent Ultra nanoLC 2D system (Eksigent Technologies, Dublin, CA) with a nano autosampler was used for liquid chromatography. The peptides were loaded on a trap column (0.3 × 5 mm, C18, LCPackings, Dionex) at 10 μL/min and the LC gradient was delivered at 300 nL/min and consisted of a linear gradient of mobile phase B developed from 2 to 40% B in 18 min on a 10 cm × 75 μm column (Reprosil AQ C18 particles, 3 μm; Dr. Maisch, Germany). The nanoLC system was connected to a hybrid triple quadrupole/ion trap mass spectrometer (6500 QTRAP, ABSciex, Foster City, CA) equipped with a nano-electrospray interface operated in the positive ion SRM mode. Parameters for declustering potential (DP) and collision energy (CE) were taken from a linear regression of previously optimized values in Skyline.[65] SRM transitions were acquired at unit/unit resolution in both the Q1 and Q3 quadrupoles with 5 ms dwell time and 3 ms pause between mass ranges, resulting in a cycle time of 1.5 s. All samples were run in a blinded fashion.

Data Analysis

All SRM data were analyzed using Skyline. The presence of multiple transitions and retention time alignment with standard peptides were manually reviewed to verify detection of the correct peptide analyte. Data were exported from Skyline for analysis and plotting. The amount of the peptide in each DBS sample was determined by calculating the ratio of the peak areas for the signature peptide to that of its labeled IS present at a known concentration.

Method Assessment

Response curves were generated in a DBS matrix. The heavy stable isotope−labeled peptides were added to the tryptic digests of DBS covering the following concentrations: 0.03, 0.13, 0.67, 3.35, and 16.77 fmol/uL. Three process replicates were prepared and analyzed at all concentration points. Repeatability was determined using two samples: (i) DBS from normal control and (ii) DBS pooled from two WD affected siblings. Complete process triplicates were prepared and analyzed on three independent days. Intraassay variation was calculated as the average CV obtained within each day. Inter-assay variation was the CV calculated from the average values of the 3 days. The stability of analytes in DBS was tested by using the same DBS card stored at room temperature and at −20 °C for 0, 3, and 7 days. For each DBS sample, samples were prepared in triplicate.

RESULTS

Selection of Target Peptides

To develop a quantitative method for the quantification of ATP7B in DBS, candidate peptides for ATP7B were screened by in silico trypsin digestion, using criteria previously described,[44] followed by BLAST searching to ensure that the sequences are unique within the human genome. These peptides were screened using the tryptic digests of HepG2 cells to empirically determine which ATP7B peptides could be best detected and quantified by LC−MS/MS. Several peptides were chosen based on the intensity of the extracted ion chromatogram and their fragmentation pattern in SRM mode. Data for the four most abundant peptides are presented in Table 1, and an example of full-MS and MS/MS spectra of ATP7B 1056−1077 is shown in Figure 1. Affinity-purified, rabbit polyclonal antibodies were generated against all four peptides. Because the sequence for ATP7B 1056 is highly hydrophobic in the N-terminal region (the first five amino acids in particular), the shortened sequence, ATP7B 1061−1077, was

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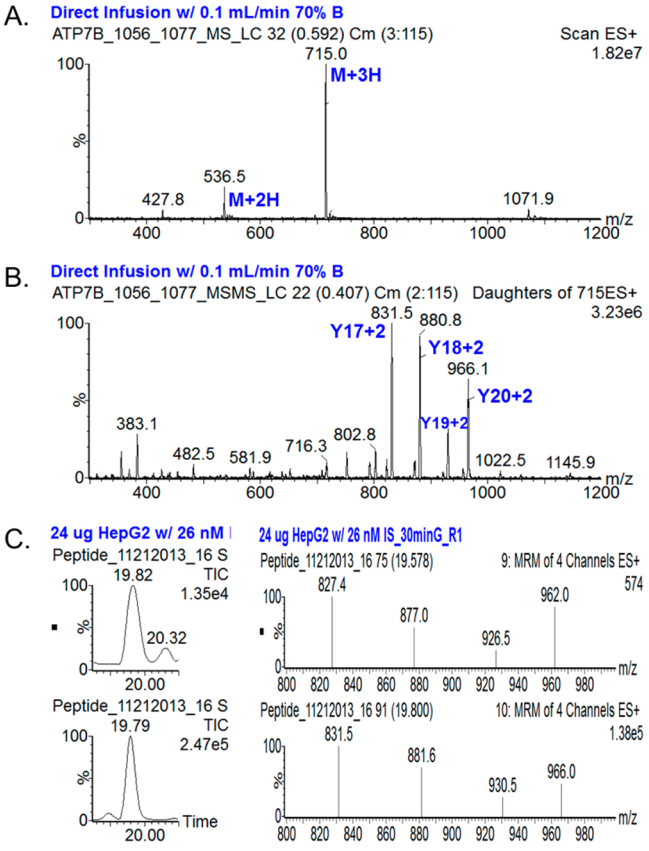


Figure 1. (A) Mass spectrum of heavy peptide 1056 for ATP7B and (B) tandem mass spectrum of the most abundant parent ion (M+3H). Abundant fragments are selected and optimized for SRM analysis. (C) Total ion chromatogram (TIC) and SRM spectra of endogenous (top) and heavy (bottom) peptide 1056 observed in HepG2 cell extract. Chromatographic peaks overlap and SRM patterns are compatible.

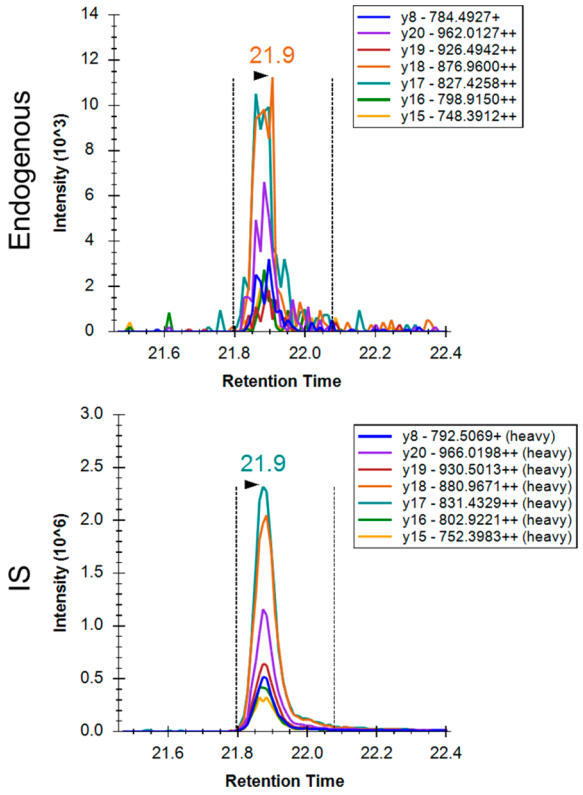


Figure 2. Extracted ion chromatograms for ATP7B 1056 peptide after peptide capture in normal PBMC. Top panel is a signature peptide found in the PBMC. Bottom panel is the isotopically labeled internal standard. Chromatographic peaks overlap, and SRM patterns are comparable. Transition labels refer to the precursor charge, fragment ion, fragment *m/z*, and fragment charge state.

used as a target for polyclonal antibody generation. While polyclonal antibodies for ATP7B 301 and 887 allowed very weak recovery of the peptides, ATP7B 325 and 1056 peptides showed recovery efficiencies ranging from ~50 to 70%. Of these two peptides, the ATP7B 1056 peptide was pursued further as a target peptide to quantify ATP7B in subsequent human samples because: (i) there was no background signal resulting from carrier peptides copurified with the antibodies[49,50,66] and (ii) the most common mutation, p.H1069Q, occurs in this peptide, taking advantage of absence of this peptide in WD patient with p.H1069Q.

Quantification of the Target Peptide by Immunoaffinity Peptide Enrichment and LC–MS/MS

While ATP7B is highly abundant in several tissues including liver, kidney, and placenta,[67] it is known to present at very low abundance in white blood cells, and it has also been observed in blood including lymphocytes and erythrocytes (gpmdb.thegpm.org). Thus ATP7B 1056 peptide was first analyzed in peripheral blood mononuclear cells (PBMCs) to see if we could identify the peptide. Isolated PBMCs were lysed and digested by trypsin, and the ATP7B 1056 immuno-SRM assay was used to enrich the peptide upstream of LC–SRM. A chromatogram of the sample eluate showing the ATP7B 1056 peptide identified in PBMC is shown in Figure 2. We next tested the feasibility of measuring ATP7B 1056 peptide in DBS. These results show the feasibility of developing an assay in DBS; we then characterized and assessed the assay for use in DBS samples.

Assay Assessment

The linearity, imprecision, and stability of the assay was assessed by following fit-for-purpose guidelines (detailed in the Experimental Procedures section).[68] The linear dynamic range was determined by generating a five-point response curve using synthetic standard peptides. Three DBS samples for each concentration level were prepared to account for any variation in protein extraction from the DBS card. These samples were analyzed in the order of increasing concentration with one blank injection between different sample concentrations. The assay showed a linear response (r^2 = 0.99) for all peptide amounts tested, spanning the peptide concentration of 27 to 16 765 pmol/L (0.7 to 417 femtomoles) (Figure 3). Results with a signal-to-noise ratio (S/N) < 10 were considered unreliable data. The low limit of quantification (LOQ) was estimated to be ~27 pmol/L based on the lowest concentration of the response curve (27 pmol/L) and patient sample no. 1 (29.5 pmol/L). The average CV was <12% for the three replicates of DBS samples prepared and analyzed at each concentration level. The linear response with the high reproducibility shows constant protein recovery and protein digestion efficiency for the target peptide. We assessed intra- and inter-assay imprecision by the LC–SRM analysis of complete process triplicates prepared from a healthy subject and a pooled patient sample and analyzed on three independent days. The results are summarized in Table 2.

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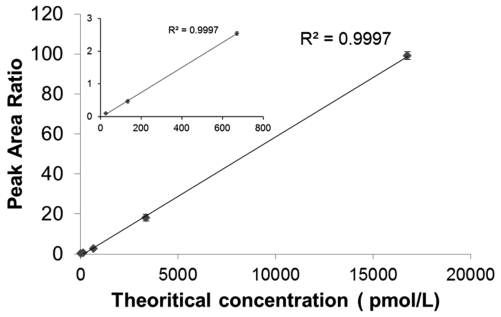


Figure 3. Response curve for ATP7B 1056 peptide. Curves are plotted for the sum of all transitions. The inset plot shows more detail of the lower end of the concentration range. Error bars are the standard deviation of three process replicates.

Table 2. Intra-Assay and Inter-Assay Imprecision of Immuno-SRM Assay for ATP7B 1056 Peptide[a]

sample	intra-assay, %	inter-assay, %
normal control	8.42	2.9
WD patient	NA	NA

[a]NA, not applicable.

Intra- and inter-assay imprecision from the normal control were 8.4% CV and 2.9% CV, respectively, suggesting the high reproducibility of both sample preparation and the method of analysis. The assay imprecision from the pooled WD patients was not calculated because most peaks observed were below the LOQ. The stability data for the ATP7B peptide is presented in Figure 4. The ATP7B peptide was stable for at least 7 days in DBS at RT and −20 °C.

Evaluation of ATP7B as a Marker for Early Screening of WD

To determine if the chosen target peptide could be used as a screening marker for WD, a total of 25 DBS samples from 12 controls and 13 confirmed WD patients were analyzed in a blinded fashion for ATP7B. Representative SRM traces

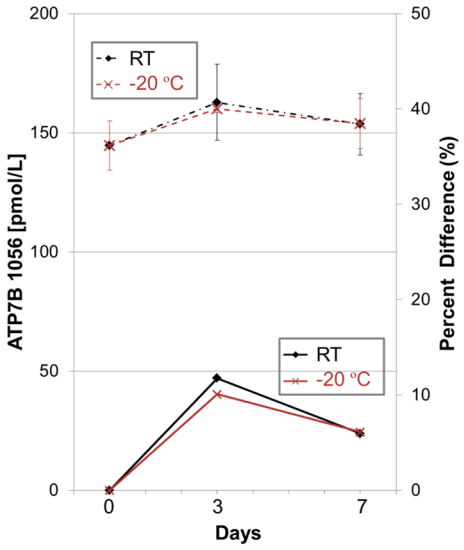


Figure 4. Stability of ATP7B 1056 peptide in normal control DBS at room temperature and −20 °C for 0, 3, and 7 days. The data represent the average of three replicates. Dashed and solid lines represent ATP7B concentrations and percent difference, respectively. Error bars are the standard deviation of three process replicates.

obtained from a healthy control and a WD patient are presented in Figure 5. The results are summarized in Table 3

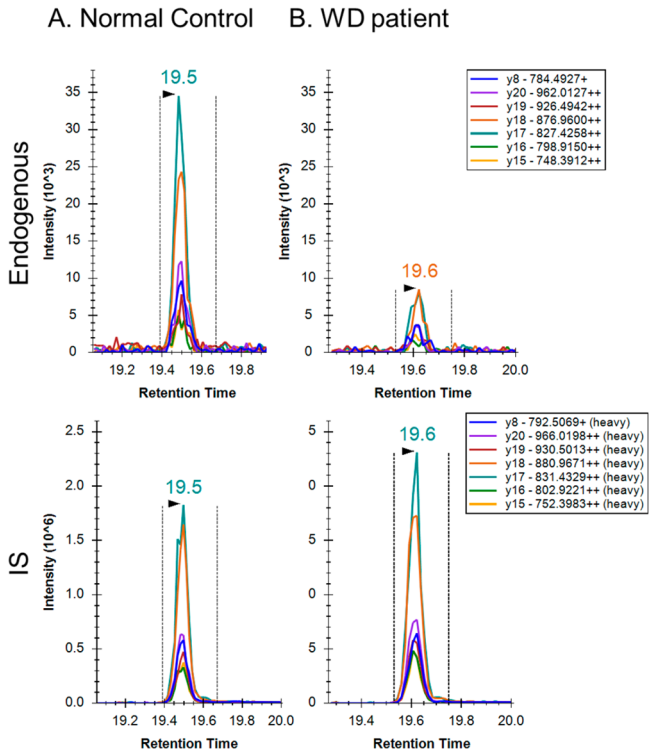


Figure 5. Extracted ion chromatograms for ATP7B 1056 peptide after peptide capture in DBS from (A) normal control and (B) WD patient. Top panel is a signature peptide found in DBS. Bottom panel is the isotopically labeled internal standard. Chromatographic peaks overlap and SRM patterns are comparable. Transition labels refer to the precursor charge, fragment ion, fragment m/z, and fragment charge state.

and Figure 6. As shown, the assay readily distinguished affected patients from controls ($p < 0.0001$). While we were able to reliably detect endogenous ATP7B ranging from 105 to 708 pmol/L in normal controls, the analyte response from WD patients was either not detected or below 29.5 pmol/L, except for one case, WD08. Of note, there was no ATP7B 1056 peptide detected in either of the WD patients who carried p.H1069Q mutation, as predicted. Case 8 presented with presumably alcoholic liver cirrhosis at the age of 56 years with a history of chronic jaundice, ascites, and hepatosplenomegaly. The copper content in the liver biopsy was marginally elevated at 116 μg/g dry weight tissue (control <35 μg/g), which prompted a genetic test for *ATP7B* gene. She was found to carry one known pathogenic variant, p.Thr974Met, and one variant of unknown significance, p.Ser391Leu in trans. Her 24 h urine copper was normal at 17 μg/24 h, serum CP was within the normal range, and no Kayser−Fleischer ring was detected in her eyes. Given her clinical and biochemical evaluations, she was suspected to be a presumtive carrier for Wilson's Disease, and the VUS was predicted to be benign in nature. Her mildly elevated copper content in the liver tissue was considered most likely secondary to long-standing liver disease.

The levels of ATP7B 1056 peptide were determined to see if the assay can distinguish between WD patients and proven carriers. Because of the high prevalence of carriers, it is important that a screening test should be able to limit or avoid

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Table 3. ATP7B 1056 Peptide Concentration in 13 DBS Samples from WD Patients

sample	ATP7B 1056 (pmol/L)	mutation
WD01	29.5	p.R778W and p.T977M
WD02	ND[a]	p.H1069Q and p.R1319*
WD03	ND	p.H1069Q and p.R1319*
WD04	ND	not available at this time
WD05	ND	p.R778L homozygote
WD06	ND	p.C2304_2305insc and p.L1083F
WD07	NA[b]	p.R778L and p.A874 V
WD08	244.8	p.Thr974Met and p.Ser391Leu
WD09	ND	p.R778G and p.K175S_fs/p.Q260P_fs
WD10	ND	p.R778G and p.K175S_fs/p.Q260P_fs
WD11	ND	p.R778L and p.E1064A
WD12	ND	p.R778L and p.E1064A
WD13	ND	p.H1069Q and p.Y1331Tfs*61
controls (N = 12)	320.9 ± 147.4	

[a]ND, not detected. [b]NA, not applicable due to S/N < 10.

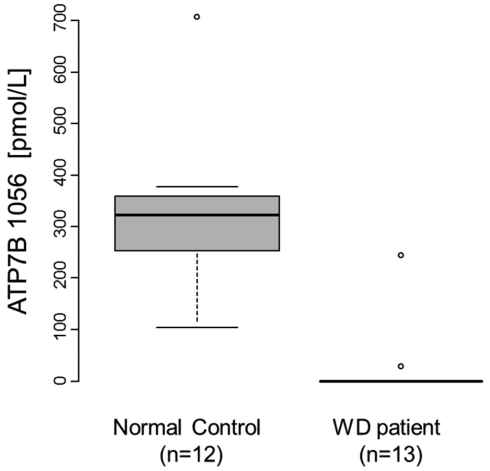


Figure 6. Distribution of the levels of ATP7B in DBS from 13 WD patients and 12 normal controls. The bold black line indicates the median, the inner quartiles are represented by boxes, and the whiskers show 95% of the data.

carrier detections. A set of DBS specimens used for this test included a WD patient, two proven carriers (the WD patient's mother and father), and two age-matched normal controls. These DBS had been stored at −20 °C for ∼15 years. The results are shown in Table 4. ATP7B 1056 peptide in the WD patient was markedly reduced compared with the two carriers and two age-matched controls. The levels of ATP7B 1056 peptide did not differ between the carriers and age-matched controls. Of note, the levels of ATP7B from the WD patient and the four controls were in the range of the WD patients and the control groups tested in this study, indicating that the protein in DBS could be stable for many years.

Table 4. ATP7B 1056 Peptide Concentration in 15 year old DBS

sample	ATP7B 1056 (pmol/L)
WD patient	60.3
Carrier 1 (mother)	270.9
Carrier 2 (father)	251.4
age-matched NC 1	369.5
age-matched NC 2	452.6

DISCUSSION

WD is a progressive and fatal disorder that is treatable, where early detection can make a significant impact on disease outcome and even be life-saving. However, there is currently no suitable marker and method available for population screening. In this study, we: (i) propose ATP7B as a potential marker to screen WD, taking advantage of an absence or decrease in the amount of the ATP7B protein in most WD patients; (ii) provide a sensitive immuno-SRM assay for the quantification of ATP7B in DBS, demonstrating the feasibility of DBS/immuno-SRM formats for screening congenital disorders lacking marker proteins; and (iii) suggest our approach can be further applied to aid diagnostics in conjunction with clinical and other biochemical test results.

We were able to reliably detect endogenous ATP7B in DBS from normal controls in the range of 105−708 pmol/L. To our knowledge, the detection and quantification of ATP7B protein in DBS has never been achieved with any method before. In 12 out of 13WD patients, the amount of the deficient ATP7B was below the control range, suggesting the feasibility of the use of ATP7B as a potential marker for WD. This approach is unique as most studies are looking for accumulated metabolites or markers, whereas this assay directly analyzes the affected protein. Although the number of samples tested in this study is quite limited, the results on the patients carrying the two most common mutations (p.R778L and p.H1069Q) are promising and demonstrate the feasibility of the immuno-SRM approach for mass screening.

The result on case 8 highlights the use of this assay for those patients with ambiguous genetic or biochemical test results, which is not an uncommon situation in the clinic. The variant of uncertain significance is not uncommon in ATP7B gene, while there is no definite diagnostic test available. The diagnosis for WD could be challenging, especially with those ambiguous or borderline results that could potentially lead to unnecessary treatment. Our result seems very promising in aiding the appropriate and accurate diagnosis in conjunction with other genetic and biochemical test results, although further studies on many clinical samples are necessary.

While the data presented in this study indicate the possible use of ATP7B in DBS for screening WD, we acknowledge that the findings are preliminary. Larger studies including both controls with proven carriers and patient samples with a broad mutational spectrum will be required to determine more

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accurate reference, disease ranges, and cutoff. In addition, patient samples tested in this study are limited to children or adults due to the difficulty of identifying newborn samples from affected patients. Although we expect no significant age dependence of its abundance, the effect of age on the level of ATP7B protein (in particular for newborns) is not known. As with all NBS assays, the implementation of this assay will need to be tested in the newborns or infants on whom the testing would be carried out.

As anticipated, there was no detectable ATP7B 1056 peptide in either of the WD patients who carried the p.H1069Q mutation that occurs within this peptide. However, it can be argued that the absence of this peptide could be due to sequence variations (mutations/polymorphisms) from presumed healthy subjects. This could be resolved by monitoring multiple peptides for each target protein, which will help ensure that negative results are truly negative. The reduced/absence of multiple peptides in a SRM assay could increase the confidence of a negative result. This applies to the quantification of any protein to prevent underestimation due to single nucleotide polymorphisms and posttranslational modifications.

Becaue of the nature of the individual variants, measurement of a target protein, ATP7B, may not be sensitive enough to identify all affected individuals. For example, some patients with mutations that affect protein function/structure but not quantity may not be detected. One approach to address this limitation is the use of additional/secondary markers such as CP. When the primary marker shows ambiguous or undetermined result, the application of secondary markers can help improve both the sensitivity and specificity of the assay. It is important to note that the ability to measure multiple analytes in the same analysis would not require additional sample process or sample collection.

DBS offers many advantages such as a less invasive sampling, a simpler storage, and an easier transfer. However, there are several variables that may affect how uniformly blood and analytes spread across the filter paper, influencing precision in quantitative analysis. These include the hematocrit values, blood volume spotted, and chromatographic effects. There are contradicting reports in the literature as to whether these variables have a direct impact on the precision of the analytical result.[69,70] Note that in our proof-of-principle study we chose to reduce the effects of these variables by spotting the same volume of blood for all DBS samples used in experiments in this study and using nearly the entire blood spots for analysis.

The performance characteristics of our immuno-SRM assay were found to be compatible with expectations for clinical sample analysis. The inter-assay and intra-assay CV of ATP7B assay using DBS from a healthy control specimen were <10%, demonstrating that the assay is precise. The response curve was linear, with an R^2 of 0.99 and the dynamic range from 27 to 16 765 pmol/L. The use of immuno-SRM also offers other compelling advantages with respect to NBS. These advantages include: (i) this assay can be multiplexed without loss of specificity and sensitivity, enabling simultaneous analysis of multiple proteins from a single sample and a single injection, permitting greater statistical power to be achieved and robust cross-correlations to be made and (ii) screening programs already have been using MS/MS technology, in particular, SRM.

DBS is an attractive alternative to the collection of plasma or serum, particularly for NBS. Analytes are known to be more stable in DBS compared with those in plasma, blood, or other solutions,[71] likely because of the dehydration of the sample on the card and consequent minimization of chemical and enzymatic hydrolysis of the analytes.[71] Note that in our bottom-up approach to quantify the protein we focused on the integrity of the targeted peptide that serves as surrogates for the protein and not the intact protein itself. Our stability test showed that the ATP7B peptide is fairly stable in DBS for a week at room temperature and −20 °C. Additionally, results from the ATP7B peptide from DBS stored at −20 °C for ∼15 years were comparable to fresh samples, suggesting good long-term stability. Our data strongly suggest that peptides in DBS may be stable, opening up the possibility of application of immuno-SRM to NBS for testing for other genetic conditions such as primary immunodeficiencies or cystinosis.

While screening programs already utilize MS/MS technology for small molecules, immuno-SRM for quantification of proteins is a novel platform in the clinical and NBS laboratories. The method for measuring proteins uses quite different procedures from measuring small metabolites. The assay requires a relatively large number of steps before LC−MS/MS: (i) proteolytic digestion of proteins in the DBS; (ii) immunoaffinity enrichment of target peptides; and (iii) MS/MS coupled to liquid chromatography. Although this is a relatively complex assay format, the implementation of robotic sample preparation for trypsin digestion and peptide enrichment and a robust chromatography configuration[45] should enable this technology to advance into routine clinical analysis for thousands of samples.

A limitation for setting up an immuno-SRM assay may be the time required and likelihood of success in generating an antipeptide antibody.[66] Monoclonal antibodies are preferable for clinical or screening assays because they provide a renewable resource for long-term supply with acceptable consistency and reproducibility. Some monoclonal antibodies outperform the polyclonal antibodies with increased recovery efficiency and sensitivity of the immuno-SRM assay.

■ CONCLUSIONS

We developed a novel, sensitive immunoaffinity LC−MS/MS assay for quantitative measurement of ATP7B levels in DBS. This study demonstrates the feasibility of the use of immuno-SRM to quantify ATP7B in DBS to screen for WD. To the best of our knowledge, this is the first published report of employing immuno-SRM strategy for measuring a clinically important, low-abundance protein in DBS. The described method opens up future opportunities for the analysis of other protein markers in DBS for many other life-threatening congenital disorders that are currently not a part of the NBS.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sihahn@uw.edu. Tel: 206-987-7647.

ORCID

Si Houn Hahn: 0000-0001-6809-5905

Notes

The authors declare no competing financial interest.

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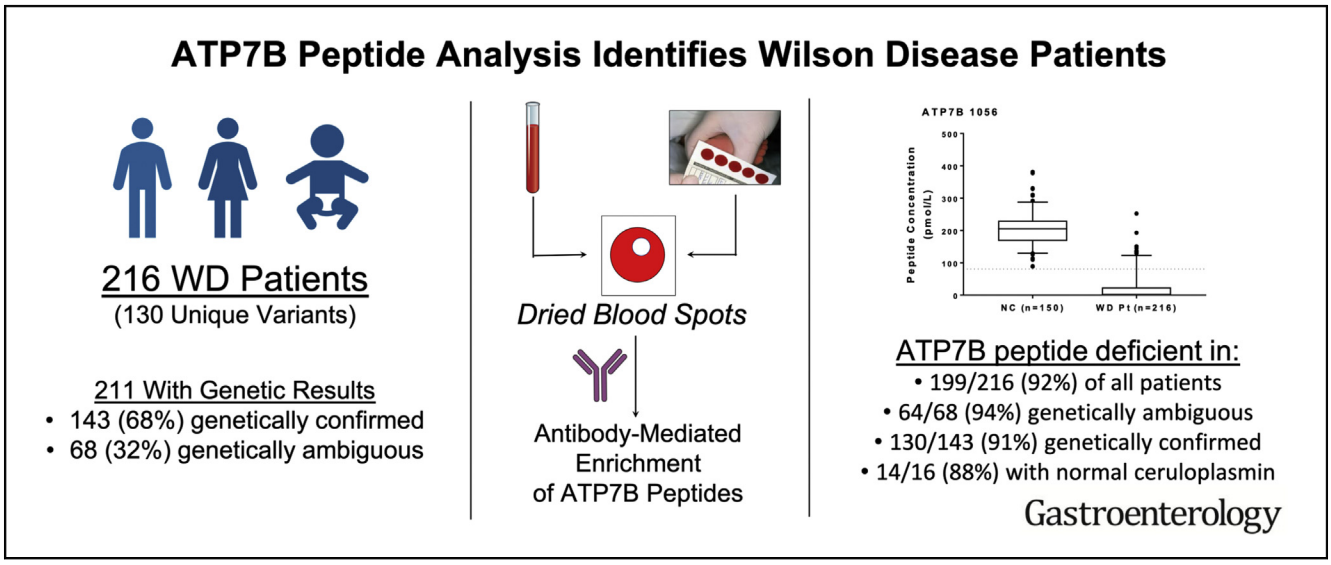
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CLINICAL—LIVER

Direct Measurement of ATP7B Peptides Is Highly Effective in the Diagnosis of Wilson Disease

Christopher J. Collins,[1] Fan Yi,[1] Remwilyn Dayuha,[1] Phi Duong,[1] Simon Horslen,[2] Michelle Camarata,[3] Ayse K. Coskun,[3] Roderick H. J. Houwen,[4] Tudor L. Pop,[5] Heinz Zoller,[6] Han-wook Yoo,[7] Sung Won Jung,[8] Karl H. Weiss,[9] Michael L. Schilsky,[3] Peter Ferenci,[10] and Si Houn Hahn[1,2]

[1]Seattle Children's Research Institute, Seattle, Washington; [2]University of Washington School of Medicine, Seattle, Washington; [3]Yale University School of Medicine, New Haven, Connecticut; [4]Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands; [5] Second Pediatric Clinic, University of Medicine and Pharmacy, Iuliu Hatieganu, Cluj-Napoca, Romania; [6]Medical University of Innsbruck, Innsbruck, Austria; [8]Gachon University School of Medicine, Incheon, Korea; [7]Asan Medical Center, Seoul, Korea; [9]Heidelberg University Hospital, Heidelberg, Germany; and [10]Medical University of Vienna, Vienna, Austria



See Covering the Cover synopsis on page 2231; See editorial on page 2249.

BACKGROUND & AIMS: Both existing clinical criteria and genetic testing have significant limitations for the diagnosis of Wilson disease (WD), often creating ambiguities in patient identification and leading to delayed diagnosis and ineffective management. ATP7B protein concentration, indicated by direct measurement of surrogate peptides from patient dried blood spot samples, could provide primary evidence of WD. ATP7B concentrations were measured in patient samples from diverse backgrounds, diagnostic potential is determined, and results are compared with biochemical and genetic results from individual patients. **METHODS:** Two hundred and sixty-four samples from biorepositories at 3 international and 2 domestic academic centers and 150 normal controls were obtained after Institutional Review Board approval. Genetically or clinically confirmed WD patients with a Leipzig score >3 and obligate heterozygote (carriers) from affected family members were included. ATP7B peptide measurements were made by immunoaffinity enrichment mass spectrometry. **RESULTS:** Two ATP7B peptides were used to measure ATP7B protein concentration. Receiver operating characteristics curve analysis generates an area under the curve of 0.98. ATP7B peptide analysis of the sequence ATP7B 887 was found to have a sensitivity of 91.2%, specificity of 98.1%, positive predictive value of 98.0%, and a negative predictive value of 91.5%. In patients with normal ceruloplasmin concentrations (>20 mg/dL), 14 of 16 (87.5%) were ATP7B-deficient. In patients without clear genetic results, 94% were ATP7B-deficient. **CONCLUSIONS:** Quantification of ATP7B peptide effectively identified WD patients in 92.1% of presented cases and reduced ambiguities resulting from ceruloplasmin and genetic analysis. Clarity is brought to patients with ambiguous genetic results, significantly aiding in noninvasive diagnosis. A proposed diagnostic score and algorithm incorporating ATP7B peptide concentrations can be rapidly diagnostic and supplemental to current Leipzig scoring systems.

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Keywords: Wilson disease; Leipzig Score; Immuno-SRM; ATP7B.

Wilson disease (WD) is named for Dr Samuel Alexander Kinnier Wilson, who first described the disorder in his 1912 doctoral thesis. Since then, treatments have been developed for WD and, importantly, WD has become a preventable disease. WD is an autosomal recessive disorder of copper metabolism due to mutations in the *ATP7B* gene that encodes copper-transporting P-type ATPase (EC # 7.2.2.8).[1–3] WD has an estimated prevalence of 1:30,000 and a carrier frequency of 1:90 with regional variation.[4,5] Although guidelines for the diagnosis of WD have been developed,[6,7] patient identification remains a challenge resulting in delayed diagnosis and development of irreversible severe complications, such as permanent brain or liver damage, which render treatments ineffective.[5]

The key features of WD are liver disease, neuropsychiatric abnormalities, and Kayser-Fleischer (KF) rings. The presence of KF rings with neurologic manifestation and/or low serum ceruloplasmin (Cp) is considered enough to establish WD diagnosis. However, most cases require a combination of clinical symptoms and laboratory evaluations.[8] Currently, no single test permits de novo WD diagnosis in every potential patient.[7] Serum Cp is decreased in neurologic WD, but can be in the low-normal range in up to 50% of adult patients with active liver disease[9,10] and the positive predictive value of serum Cp for diagnosis of WD is poor.[9,11,12] In children with WD, 15%–36% had Cp in the normal range.[13] Serum Cp alone is not sufficient to diagnose or exclude WD. A diagnostic score (Leipzig score, 2003) was proposed to guide clinical diagnosis and has been adopted in the clinical practice guidelines for the European Association for the Study of the Liver.[14] Although recent advances in clinical molecular diagnosis have greatly improved the accuracy of WD diagnosis in affected patients and their siblings, traditional Sanger sequencing cannot detect large deletion or duplications. In addition, there are many single nucleotide polymorphisms and variants of unknown significance (VUS) in the ATP7B gene. Interpretation of genetic sequencing results, particularly in the presence of only one identified mutation or VUS, create ambiguity in WD patient identification.

We evaluated the direct measurement of ATP7B protein from WD patient dried blood spots (DBS), through surrogate ATP7B peptides, as a diagnostic tool.[15] As reported in our previous studies for multiple primary immunodeficiency conditions, peptide measurements are made using immunoaffinity enrichment coupled to selected reaction monitoring (immuno-SRM) mass spectrometry.[16,17] This method uses antipeptide antibodies to concentrate and quantify extremely-low-concentration peptide targets from complex matrices, including DBS.[15–21] Analysis of ATP7B concentration in DBS from WD patients with a broad range of genetic backgrounds shows that ATP7B peptide levels are greatly reduced. Analysis of ATP7B protein concentration can identify WD with high diagnostic accuracy.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Identification of patients with Wilson disease remains a challenge, resulting in delayed diagnosis and development of irreversible severe complications, such as permanent brain or liver damage, which render treatments ineffective.

NEW FINDING

Directly measuring ATP7B from dried blood spots of patients with Wilson disease with diverse genetic backgrounds showed ATP7B peptide concentrations have a high diagnostic potential.

LIMITATIONS

The study is limited in that data are mostly obtained from White patients. Sensitivity and specificity may be variable geographically.

IMPACT

ATP7B peptide analysis identifies most patients with Wilson disease, reducing ambiguities resulting from genetic analysis, and is expected to advance the use of proteomics, a promising but largely clinically untapped technology.

Methods

Dried Blood Spot Samples

This protocol was approved by the Institutional Review Board of Seattle Children's Hospital (SCH) and each of the participating institutes. All subjects gave written informed consent. Patient and carrier samples were provided by SCH, Seattle, WA; Medical University of Vienna, Austria; Medical University Innsbruck, Austria; University of Medicine and Pharmacy, Iuliu Hatieganu, Cluj-Napoca, Romania; Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands; Yale University, New Haven, CT; University of Heidelberg, Germany; and Asan Medical Center, Seoul, South Korea. Samples were prepared either by fingerstick or by pipetting 70 μL of blood (per 12-mm spot) onto filter paper cards (903 ProteinSaver; Whatman, Piscataway, NJ). The samples were then dried overnight at room temperature, delivered to SCH, and stored at −80°C until use. One hundred and fifty normal control DBS samples (BioIVT, Westbury, NY) were analyzed to establish the normal reference range and cut-off.

Abbreviations used in this paper: **ACN, acetonitrile; AF, allele frequency; CHAPS, 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate; Cp, ceruloplasmin; CV, coefficient of variation; DBS, dried blood spot; FA, formic acid; immuno-SRM, immunoaffinity enrichment coupled to selected reaction monitoring; IS, internal standard; KF, Kayser-Fleischer; LC/MS, liquid chromatography/mass spectrometry; LLOD, lower limits of detection; LLOQ, lower limits of quantification; mAb, monoclonal antibody; SCH, Seattle Children's Hospital; VCI, variant with conflicting interpretations; VUS, variants of unknown significance; WD, Wilson disease; WT, wild-type.**

Most current article

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Immunoaffinity Enrichment Coupled to Selected Reaction Monitoring Reagents

Triton X-100 (T9284, 100 mL) and Ammonium bicarbonate (A6141-25G) were purchased from Sigma-Aldrich (St. Louis, MO). TPCK-treated Worthington trypsin (LS003740) was purchased from Worthington (Lakewood, NJ). 3-([3-cholamidopropyl]dimethylammonio)-1-propanesulfonate (CHAPS) (no. 28300), acetonitrile (ACN) (liquid chromatography/mass spectrometry [LC/MS] grade), acetic acid (LC/MS grade), water (Optima LC/MS grade), formic acid (FA) (Optima LC/MS grade), 1× phosphate-buffered saline (no. 10010-023), dithiothreitol (no. 20290), and 1M Tris-(hydroxymethyl)aminomethane (pH 8) (no. 15568-025) buffer were obtained from Fisher Scientific (Waltham, MA). Protein G–coated magnetic beads (Dynabeads, no. 10004D) were purchased from Invitrogen (Carlsbad, CA).

Isotope-labeled internal standard (IS) peptides were purchased from either Atlantic Peptides (Lewisburg, PA) or Life Technologies Corporation (Carlsbad, CA). IS peptides were >95% pure and incorporated heavy stable isotope-labeled (^{13}C and ^{15}N) C-terminal lysine (+8 Da) or arginine (+10 Da). IS stock solutions are stored frozen as 500× mixtures in 1× phosphate-buffered saline + 15% ACN + 0.1% FA + 0.03% CHAPS in H_2O and diluted to 1× immediately before use.

Selection of Signature Peptides and Antibody Production

Selection and production of ATP7B 1056 peptide and antibody has been described in previous reports.[15–17]

Using the same guidelines, ATP7B 887 was selected. Antibody production was performed by Excel Biopharm (San Francisco, CA), Pacific Immunology (Ramona, CA), and ExonBio (San Diego, CA). Enzyme-linked immunosorbent assays were performed, by the companies mentioned, in bleed samples after immunization and in supernatant samples after monoclonal antibody production. Selections were further confirmed at each step by the immuno-SRM method.[22]

Antibody Bead Reagent Production

Monoclonal antibody (mAb) beads were produced by overnight 4°C incubation of Protein G Dynabeads with mAb, as reported previously.[15–17]

Dried Blood Spot Extraction, Trypsin Digestion, and Immunoaffinity Enrichment

Protein extraction and tryptic digestion were performed as reported previously, with slight modifications.[16] One 6.35-mm diameter DBS punch was placed into 96-well plates (ThermoScientific, Chicago, IL), covered with an adhesive seal (Genesee Scientific, San Diego, CA), and extracted using 0.1% Triton X-100 in 50 mM ammonium bicarbonate (200 μL) with dithiothreitol (final concentration 0.2M). After 30 minutes incubation at 37°C with agitation, trypsin (37.5 μg) was added and incubated for 2 hours at 37°C. For enrichment, 10 μL of 1M TRIS (pH 8) and 10 μL of 1× ATP7B IS mix were added (final concentrations = 0.25 nM). Extracted supernatant (200 μL) was transferred to a new plate and incubated with 2.5 μL of each mAb bead overnight at 4°C with agitation.

After incubation, mAb beads were isolated using a 96-well magnetic plate (Alpaqua Magnum EX, Beverly, MA), washed twice with 1× phosphate-buffered saline + 0.01% CHAPS, and magnetically isolated. Peptides were eluted with 30 μL of H_2O with 5% acetic acid and 3% ACN for 5 minutes and transferred to a new 96-well plate for analysis (Abegene, Chicago, IL).

Liquid Chromatography-Mass Spectrometry

LC-MS/MS was performed using a Waters Xevo TQ-XS with Ionkey source and dual M-Class chromatography pumps (Milford, MA). Chromatographic solvents were A: H_2O + 0.1% FA and B: ACN + 0.1% FA. Peptides are loaded onto an M-Class Trap Symmetry C18 column (300 μM x 25 mm, 100A, 5 μM) for 3 minutes with a constant flow of 98:2 A:B at 20 μL/min. After loading, the flow is reversed and peptides are separated using a 150 μM × 100 mm BEH C18 ionkey (130 Å, 1.7 μM). The gradients used are summarized in Supplementary Table 1 and were reported previously.[16] Precursor mass, fragment mass, and collision energy were tuned to optimize the generated signal (Supplementary Table 2). Representative chromatograms for both ATP7B 887 and ATP7B 1056 peptides were shown in Supplementary Figure 1*A–D*.

Concentration Calculation and Data Analysis

Selected reaction monitoring data captured in the MS were analyzed using Skyline (MacCoss Lab, Seattle, WA, https://skyline.ms/project/home/begin.view).[23] Specificity was assured by monitoring retention times and relative transition intensities of endogenous and IS peptides. Concentrations of endogenous signature peptides were calculated using endogenous/IS signal ratio. DBS spots are assumed to contain 70 μL of evenly distributed whole blood. The volume of blood in the punch area is calculated as 17.5 μL. Concentrations are calculated from blood volume, ratio, and IS concentration. Statistical analyses and receiver operating characteristics curves were generated using GraphPad Prism (San Diego, CA).

Method Performance Assessment

Response curves were generated for each peptide to establish assay linearity and determine the lower limits of detection (LLOD) and quantification (LLOQ). Seven concentrations (0×, 0.05×, 0.1×, 0.5×, 1×, 5×, and 50×) of IS were added across the set of pooled digest samples in triplicate. LLOD was calculated as: $LLOD = mean_{blank} + 3 \times SD_{Low}$ ($mean_{blank}$: mean signal from a triplicate blank injection, SD_{Low}: SD of IS injection below the LLOQ). LLOQ is the lowest concentration with a coefficient of variation (CV) of <20%. The linearity curves of ATP7B 1056 and ATP7B 887 are shown in Supplementary Figure 1*E* and *F*.

The assay precision and accuracy were evaluated by within-day (intra-) and between-day (inter-) assay CV, respectively. The intra- and inter-assay CV were determined using 5 replicates of an identical pooled blood DBS sample each day over a course of 5 days (Table 1).

Internal Sample Quality Control

Measurement of endogenous peptides unrelated to WD and, therefore, assumed to be present at normal concentrations, was used as an internal quality control to monitor the successful

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Table 1. Analytical and Diagnostic Performance for ATP7B Peptides

Peptide	LLOD, *pmol/L*	LLOQ, *pmol/L*	Intra-assay CV, %	Inter-assay CV, %	PPV, %	NPV, %	AUC
ATP7B 1056	3.81	71.43	12.9	15.3	96.1	91.3	0.98
ATP7B 887	2.17	7.14	11.0	13.0	98.0	91.5	0.98

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

extraction, digestion, and enrichment of target peptides. These peptides are ADA 93, representing adenosine deaminase, CD42 128, representing glycoprotein Ib, and IDUA 462, representing α-L-iduronidase.[16] A sample run was assumed to be of sufficient quality if the measured concentration of 2 of these 3 peptides was within 1.75 SD of the mean for the cohort (Supplementary Tables 3–5). Samples failing these acceptance requirements were repeated. With confirmatory test failure, the DBS was removed from the sample cohort due to inadequate matrix and an additional sample was requested.

Results

Characteristics of Patient Cohort

WD DBS samples were obtained from 198 White and 18 Far Eastern Asian patients with WD (114 male and 102 female) and 48 obligate carriers (Supplementary Tables 3 and 4). All carriers are obligate heterozygote from the family members of index case patients with 2 confirmed variants. Patient age range spanned from 2 months to 73 years. For the purpose of stability validation, samples from 11 patients and 1 healthy normal subject were collected from both fresh and blood samples stored up to 11 years prior (Supplementary Table 7). Clinical information including Cp concentrations, Leipzig scores, liver copper content, presence of KF rings, initial presentation, and presence of cirrhosis are presented where available (Supplementary Table 3). Control DBS samples (n = 150) were obtained from healthy subjects ranging from 18 to 73 years of age.

An analysis of the specific variants in the sample set showed that the cohort contained 130 unique variants (Figure 1*B*, Supplementary Table 6), including 83 pathogenic or likely pathogenic variants, 43 VUS, 3 benign or likely benign variants, and 1 with conflicting interpretations (VCI). In affected patient samples, 143 exhibited only pathogenic or likely pathogenic mutations according to a public database (ClinVar, gnomAD [Genome Aggregation Database]), including 31 patients homozygous for p.H1069Q, the most common variant in WD patients (Figure 1*A*, Supplementary Table 3). In addition, 20 patients were homozygotes for other variants. Seven patients exhibited 2 VUS (2 of them are homozygotes). In addition, 37 patients were compound heterozygous for 1 VUS and 1 pathogenic or likely pathogenic mutation. Eighteen patients had only 1 variant detected by Sanger sequencing. No second variant was detected. Three clinically suspected patients were compound heterozygotes, with 1 likely benign variants according to gnomAD and the second variant with likely pathogenic, pathogenic, and unknown, respectively.

Forty-eight samples from obligate carriers, all of them family members of affected patients, presented with 2 variants, had a single pathogenic variant and a single wild-type allele or a benign variant (Supplementary Table 4).

Surrogate Peptide Markers for ATP7B

The mean ± SD signature peptide concentration in normal control was 257.7 ± 57.5 pmol/L for ATP7B 887 (range, 136.4–447.0 pmol/L; 5^{th}–95^{th} percentile range, 165.0–359.6 pmol/L) and 203.0 ± 48.9 for ATP7B 1056 (range, 88.2–381.3 pmol/L; 5^{th}–95^{th} percentile range, 129.7–287.3 pmol/L). These cut-offs were set at –2.5 (114.0 pmol/L) and –2.5 SD (80.8 pmol/L) below the mean normal concentration for ATP7B 887 and ATP7B 1056, respectively.

Analytical Performance

The analytical figures of merit for immuno-SRM quantification of ATP7B peptides are given in Table 1. The LLODs of ATP7B quantification were determined to be 3.81 pmol/L and 2.17 pmol/L for ATP7B 1056 and ATP7B 887,

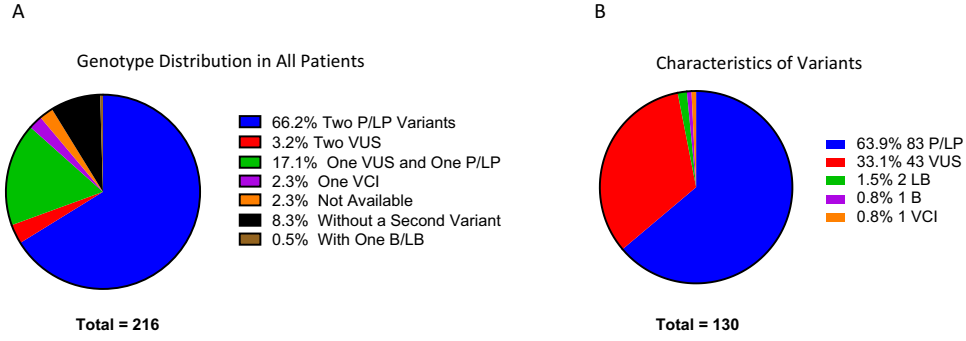


Figure 1. Patient cohort characteristics. Analysis of the genotypes of patients (*A*) and the characteristics of the variants present (*B*) show the diversity of variants and variant combinations present.

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respectively. LLOQs were determined to be 71.43 pmol/L and 7.14 pmol/L. The intra-assay CVs were 12.9% and 11.0% for ATP7B 1056 and ATP7B 887. The inter-assay CVs were 15.3% and 13.0%, respectively.

ATP7B Concentration Measurements and Primary Diagnostic Performance

Signature peptide levels in patient DBS were below cut-off in 195 of 216 (90.3%) of samples for both ATP7B 1056 and ATP7B 887 (Figure 2*A* and *B*). There were 17 WD patients (7.9%) that had ATP7B level above the cut-off for both ATP7B peptides. There were 2 WD patients had only ATP7B 887 and 2 WD patients had only ATP7B 1056 levels above the cut-off. In all, 199 of 216 patients (92.1%) had at least 1 peptide below cut-off. In WD carriers, 8 of 48 (16.7%) and 4 of 48 (8.3%) samples were below diagnostic cut-offs for ATP7B 1056 and ATP7B 887, respectively. These patient samples generate potential false positives.

As a primary diagnostic, receiver operating characteristics curve analysis (Figure 2*C* and *D*) constructed from this DBS sample cohort found that both ATP7B 1056 and ATP7B 887 peptide analysis have an area under the curve of 0.98 (ATP7B 1056 [SE = 0.006; 95% confidence interval, 0.97–0.99; $P < .0001$] and ATP7B 887 [SE = 0.007; 95% confidence interval, 0.96–0.99; $P < .0001$]). ATP7B 887 analysis was found to have a sensitivity of 91.2%, specificity of 98.1%, positive predictive value of 98.0%, and a negative predictive value of 91.5%. ATP7B 1056 showed positive predictive value of 96.1% and negative predictive value of 91.3% (Table 1).

Effects of Common Variants

In the cohort of 216 patients with WD, a total of 130 variants were identified (Supplementary Table 6). Many common pathogenic variants, including p.H1069Q (allele frequency [AF], 0.103%), p.R778L (AF, 0.013%), p.M645R (AF, 0.047%), and p.E1064A (AF, 0.015%), were associated with either an undetectable or significantly reduced level of ATP7B (Figure 3*A*–*D*).

Variants Leading to Potential False Negatives

In this cohort, 17 of 216 patients (7.9%) have ATP7B concentrations above the cut-off for both signature peptides. The genetic information is summarized in Table 2. In these 17 WD patients, 13 variants were commonly involved and could contribute to normal levels of ATP7B. According to gnomAD (https://gnomad.broadinstitute.org), these variants are rare, with an AF <0.0089%; the remaining variant, p.M665I, has a VCI designation. Of note, the 4 variants above (p.R616W, p.G710S p.M769V, and p.R969Q) have been reported to show the ATP7B protein distribution similar to wild-type (WT) in an in vitro study (Figure 3*E* and *F*).[24]

ATP7B Analysis and Variant Pathogenicity

Of the 216 WD patients, 211 had genetic test results available (Figure 2*E*). One hundred and forty-three were genetically confirmed to be WD patients by being compound heterozygous or homozygous for known pathogenic or likely pathogenic mutations (Figure 1*A*). One hundred and thirty of these patients (91.0%) had concentrations of at least 1 signature peptide below established cut-offs (Figure 4*D*). Alternatively, 68 patients had ambiguous genetic test results preventing straightforward genetic identification. Sixty-four (94%) of these patients were deficient in ATP7B by peptide analysis (Figure 2*E*). Seven patients were compound heterozygous or homozygous for 2 VUS. All samples (100%) contained significant reductions in ATP7B peptides (Table 2). Thirty-seven patients were compound heterozygous for 1 VUS and 1 known pathogenic or likely pathogenic variant (Table 2, Figure 4*E*). ATP7B concentrations were below cut-off in 35 of 37 (94.6%) of these cases. One VCI, p.M665I, was found in 5 patients. In this case, 3 patients had peptide concentrations below established cut-offs and the remaining 2 were compound heterozygous p.G710S, known to cause false negatives. Three patients had known benign or likely benign in combination with known pathogenic or likely pathogenic mutations. Two are likely carriers with normal ATP7B, but 1 patient with a likely benign mutation in combination with a known pathogenic mutation had nondetectable ATP7B, indicating possible misannotation (#64). Finally, in 18 WD cases that have only 1 variant with no second mutation detected, their ATP7B peptide levels were all (100% of samples) reduced below the cut-off (Table 2, Figure 4*F*).

ATP7B Analysis and Ceruloplasmin Concentration

The 200 patients for whom Cp values were provided were stratified into the following 3 subgroups: 107 patients with Cp <10 mg/dL, 77 patients with Cp between 10 and 20 mg/dL, and 16 patients with Cp >20 mg/dL (Figure 4*A*–*C*). Within these groups, 101 of 107 (94.3%) with Cp <10 mg/dL, 70 of 77 (90.9%) with Cp between 10 and 20 mg/dL, and 14 of 16 (87.5%) with Cp >20 mg/dL had DBS ATP7B peptide concentrations below diagnostic cut-offs.

ATP7B Analysis and Other Clinical Indications

Where possible, clinical information including age, Leipzig scores, liver copper content, presence of KF rings, initial presentation, and presence of cirrhosis are presented (Supplementary Table 3). The exact number of treated patients and their regimens is unknown. No differences in ATP7B concentration were found among the patients based on any of these factors. Fifty-nine patients had hepatic copper measurements available (Figure 4*G*–*I*). Fifty-one patients (86.4%) had elevated liver copper (>250 μg/g), 46 of them (90.2%) had deficient ATP7B levels. Eight patient had liver copper that ranged from 25 to 248 μg/g, 7 of them with deficient ATP7B levels. Of note, 3 samples were received with suspicion of WD by elevated liver copper, but with no ATP7B variants identified, indicating they are not WD patients. As expected, these samples had normal ATP7B concentrations and were not contained within the final 216 WD patient cohort.

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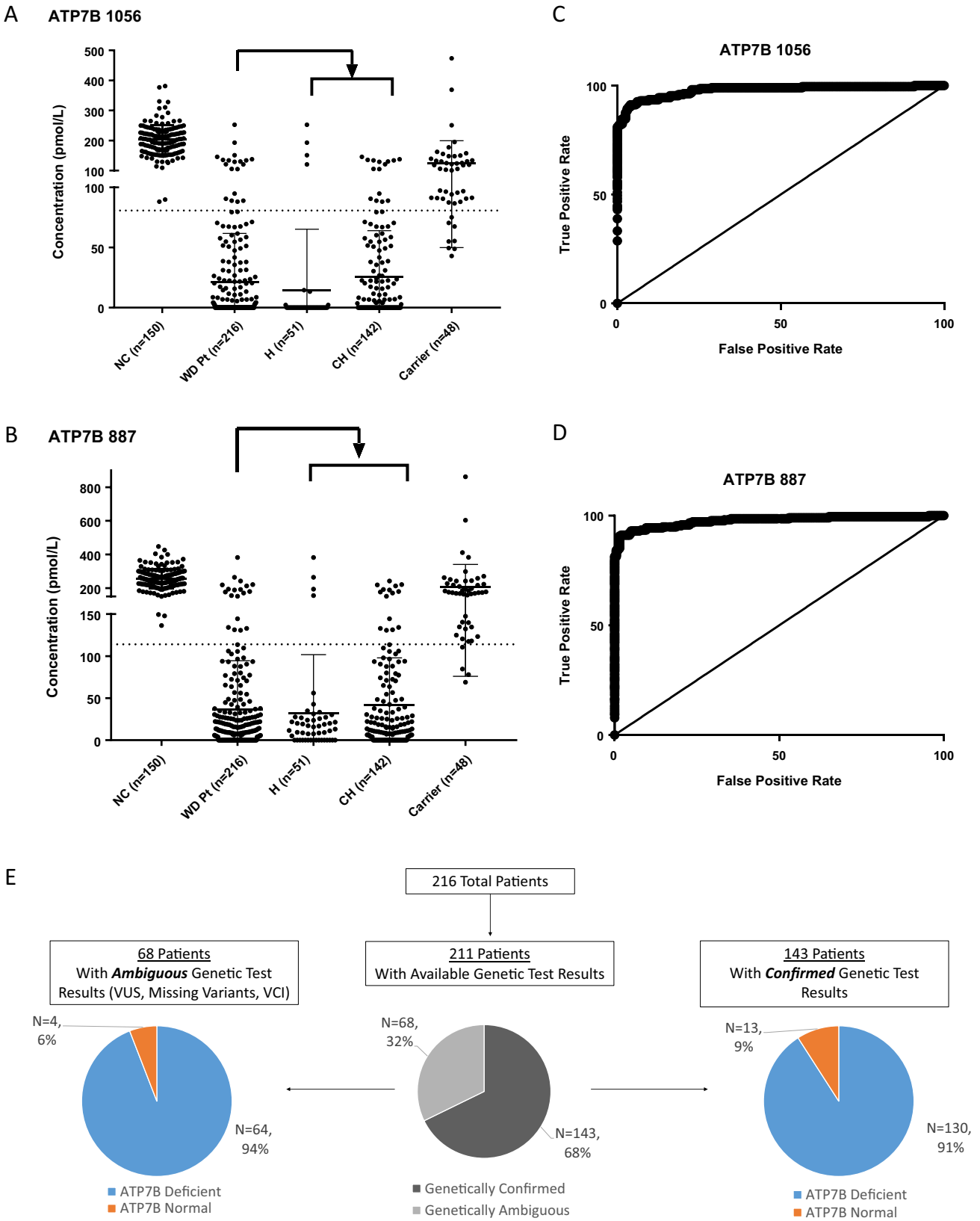


Figure 2. Diagnostic performance of ATP7B peptide analysis. Comparison of ATP7B peptide measurements for ATP7B 1056 (*A*) and ATP7B 887 (*B*) peptides in normal control patients (NC), patients, homozygotes (H), compound heterozygotes (CH), and carriers. *Dotted lines* represent diagnostic cut-offs for each peptide. Receiver operating characteristics curves show the diagnostic performance of ATP7B 1056 (*C*) and ATP7B 887 (*D*). WD patients with genetic test results are readily identified even in subgroups where genetic results are ambiguous (*E*).

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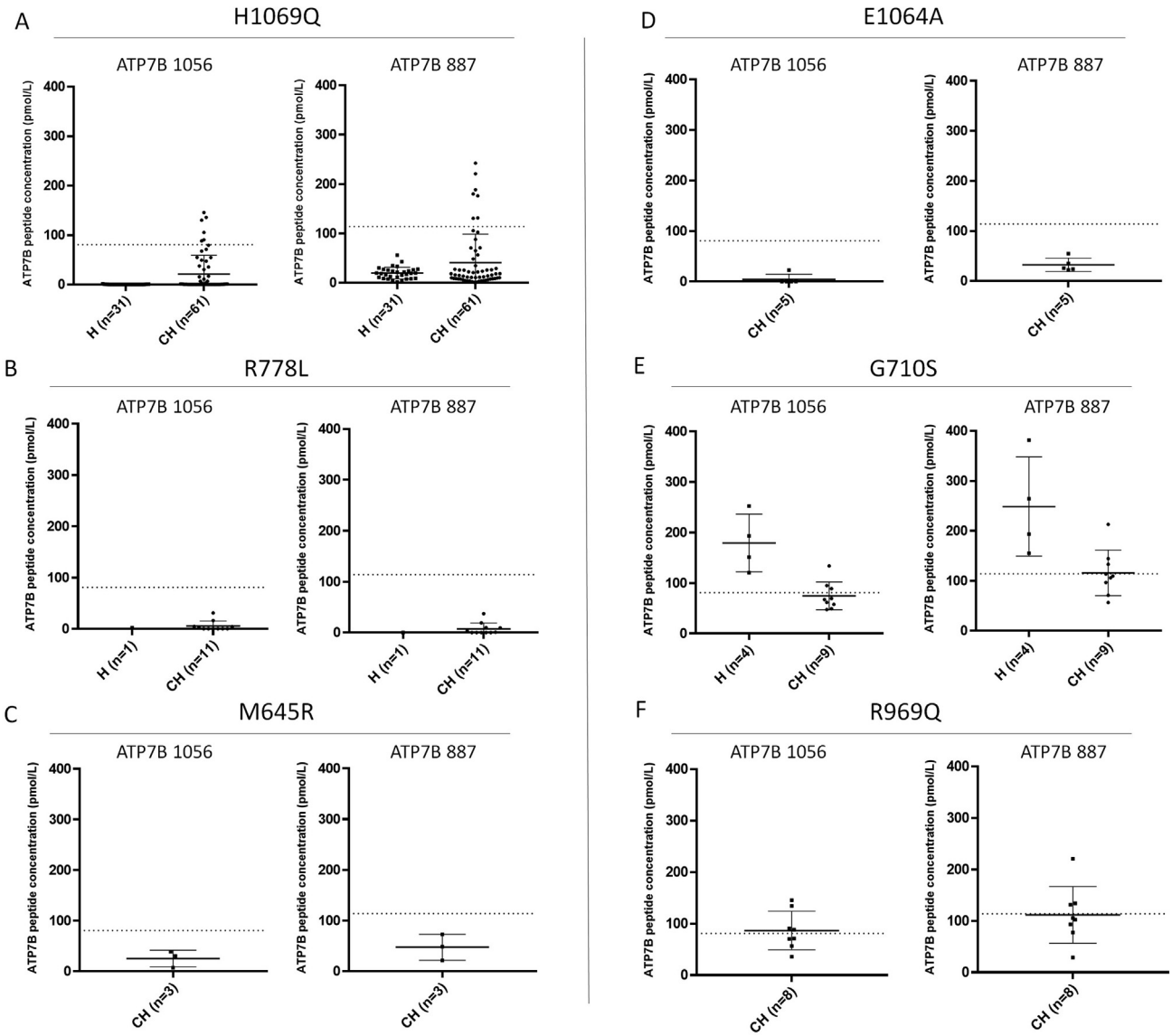


Figure 3. ATP7B peptide concentrations in patients with common variants are often reduced and variants causing false negatives are rare. Patients homozygous and heterozygous for p.H1069Q (*A*), R778L (*B*), M645R (*C*), and E1064A (*D*) are largely reduced. False negatives within the patient cohort are possible with the presence of specific variants, including G710S (*E*) and R969Q (*F*). Patients can have variable peptide concentrations depending on the second variant. CH, compound heterozygote; H, homozygote. *Dotted lines* represent peptide diagnostic cut-offs.

Discussion

This is the first large cohort study directly measuring ATP7B from DBS of WD patients with diverse genetic backgrounds. It showed ATP7B peptide concentrations have a high diagnostic potential. This test is a novel approach to WD testing and screening with a high sensitivity and specificity. It can be applied successfully in patients who do not present with clear clinical and laboratory criteria for WD as a first "second"-line test and expand the clinician's ability to noninvasively diagnose WD by reducing the need for liver biopsy. As the assay measures the ATP7B peptides produced in peripheral blood, liver synthetic dysfunction would have little or no effect on the concentration of ATP7B peptides in DBS, which is another advantage of the assay. In suspected cases, no single test is diagnostic, and a combination of laboratory tests and clinical investigation is required to establish the diagnosis. These include ophthalmologic testing for KF rings, Cp measurement, 24-hour urine copper measurement, liver biopsy to determine copper content, and *ATP7B* gene sequencing. In patients with hepatic WD, KF rings may be absent and Cp in the low to normal range can contribute to diagnostic ambiguity.

Genetic sequencing can give a definitive diagnosis when 2 known pathogenic variants are found. However, many mutations found in the *ATP7B* gene are VUS, VCI, or extremely rare. Now, more than 1300 variants in the ATP7B gene are listed in Varsome (varsome.com)[25]; 649 of them were pathogenic or likely pathogenic, and 692 of them were classified as VUS. Variant interpretation remains a challenge

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Table 2. Selected Patients From Cohort

Patient/ gender/age	Variant 1	Annotation 1	Variant 2	Annotation 2	ATP7B 1056 (pmol/L)	ATP7B 887 pmol/L	CPL (mg/dL)	Leipzig score	Liver Copper (ug/g Tissue)	KF Ring	Presentation
False negative (n=17)											
110/F/17	p.G710A	Path	p.G710S	Path	88.9	133.0	14	7	-	NP	-
10/M/34	p.G710S	Path	p.G710S	Path	120.4	155.3	<9	8	-	Y	N
11/M/17	p.G710S	Path	p.G710S	Path	193.2	264.3	12	10	-	Y	B*
12/F/44*	p.G710S	Path	p.G710S	Path	151.1	193.6	9.1	11	164	Y	N
13/M/19	p.G710S	Path	p.G710S	Path	252.4	381.7	20.7	8	1243	Y	B
111/M/47	p.M665I	Conflicting	p.G710S	Path	133.9	213.0	15.5	7	324	NP	H
112/M/NA	p.M665I	Conflicting	p.G710S	Path	94.9	144.4	NA	6	324	NP	H
140/F/29	p.R616Q	Path	p.L1305P	Path	105.5	151.7	2	8	-	Y	B
149/F/17	p.H1069Q	Path	p.M769V	Likely Path	130.2	180.1	10	8	2047	NP	H
150/F/31	p.N41S /p.I1021V	Likely Path	p.M996T	Likely Path	129.1	218.6	4	6	>250	NP	H
153/F/37	p.H1069Q	Path	p.P1273L	Path	105.3	188.5	8	10	-	Y	N
154/F/18	p.M769H-fs	Path	p.P1273L	Path	121.4	170.4	5	10	-	Y	N
170/M/NA	p.H1069Q	Path	p.R969Q	Path	90.5	131.4	NA	4	-	-	-
124/M/17	p.Q7D-fs*14	VUS	p.H1069Q	Path	135.8	242.5	19	5	-	NP	H
171/F/9	p.H1069Q	Path	p.R969Q	Path	145.6	220.9	16	5	-	NP	H
176/M/NA	p.R616W	Path	p.R969Q	Path	134.2	134.2	25	7	-	NP	H
183/F/31	p.T977M	Path	p.T991A	VUS	137.9	191.7	12	3	-	Y	H
Patients with one pathogenic/likely pathogenic variant in combination with one VUS (n=37)											
124/M/17	p.H1069Q	Path	p.Q7D-fs*14	VUS	135.8	242.5	19	5	-	NP	H
125/M/9	p.H1069Q	Path	p.Q7D-fs*14	VUS	79.4	176.3	16	5	-	NP	H
131/F/13	p.H1069Q	Path	p.I1007T-fs	VUS	ND	5.4	14	5	-	Y	H
55/M/59	p.H1069Q	Path	arr[GRCh37] 13q14.3 (52541594_52548863)x1	VUS	ND	16.1	13	5	-	Y	H
87/F/19	p.H1069Q	Path	p.D1447G-fs	VUS	67.5	130.7	13	6	-	NP	H
159/M/40	p.R827W	Likely Path	p.R1320T	VUS	58.7	93.4	13	7	-	NP	N
133/M/18	p.H1069Q	Path	p.K1028S-fs	VUS	ND	3.9	12	7	842	NP	H*
183/F/31	p.T977M	Path	p.T991A	VUS	137.9	191.7	12	3	-	Y	H
164/F/NA	p.H1069Q	Path	p.R778P	VUS	ND	ND	11	9	1332	NP	H

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Table 2. Continued

Patient/ gender/age	Variant 1	Annotation 1	Variant 2	Annotation 2	ATP7B 1056 (pmol/L)	ATP7B 887 pmol/L	CPL (mg/dL)	Leipzig score	Liver Copper (ug/g Tissue)	KF Ring	Presentation
118/M/6	p.H1069Q	Path	p.G1011X	VUS	ND	10.0	10	6	-	NP	H
157/F/8	p.R1041W	Likely Path	p.D765Y	VUS	6.8	20.7	10	8	1211	NP	H
163/M/2 mo	p.R778L	Path	p.V1106I	VUS	4.0	9.7	10	-	-	NP	A
182/M/40	p.T850I	Likely Path	p.G515S	VUS	11.6	13.4	10	6	-	Y	B
67/F/17	c.2865+1G>A	Path	p.Ser135*	VUS	ND	ND	<13	9	-	NP	H
167/F/19	p.H1069Q	Path	p.R919L	VUS	3.6	22.1	9	10	-	Y	N
95/M/53	p.H1069Q	Path	p.F1343dup	VUS	ND	ND	9	3	-	Y	H
108/M/15	p.H1069Q	Path	p.G1341E	VUS	ND	10.4	6.7	8	-	Y	H
181/F/41	p.G1266R	Path	p.T807I	VUS	8.3	5.9	6	9	291	NP	H
193/F/15	p.S1365C-fs*12	Path	p.Y743I-fs*19	VUS	ND	ND	<6	7	-	NP	H
80/M/40	p.A874V	Path	c.2299InsC	VUS	6.0	10.5	5.5	10	1575	Y	H*
120/F/13	p.H1069Q	Path	p.Lys269*	VUS	ND	ND	5	5	923	NP	H
142/M/39	p.T977M	Path	p.L1350P	VUS	22.6	32.6	5	10	-	Y	B
76/M/5	p.A1018V	Path	p.E458X	VUS	26.4	47.5	4.1	8	793	NP	H
141/F/46	p.H1069Q	Path	p.L1333P	VUS	ND	6.3	4	7	-	Y	H
184/M/10	p.A874V	Path	p.V1106I	VUS	20.1	15.5	4	-	-	-	-
119/M/38	p.H1069Q	Path	p.K844E-fs*10	VUS	ND	8.7	<4	4	-	NP	N
79/M/59	p.L1088X	Likely Path	p.A1135Q-fs*13	VUS	ND	ND	<4	6	-	Y	H
165/M/15	p.R778W	Path	p.K35N-fs*6	VUS	21.8	25.1	<3	4	-	NP	H
143/F/26	p.R778L	Path	p.L770L	VUS	ND	ND	<3	9	-	Y	H
69/F/16	p.T1029I	Path	c.3060+5G>C	VUS	2.8	ND	<3	8	-	Y	H
74/F/12	p.H1069Q	Path	IVS19-1C>G	VUS	ND	13.5	3	9	-	Y	N
188/F/8	p.M645R	Path	p.V997-fs	VUS	38.7	72.4	2.4	8	>250	NP	H*
102/M/9	p.G1341D	Path	p.F1026F-fs	VUS	ND	ND	2	10	-	Y	N
134/F/10	p.M769H-fs	Path	p.K1028S-fs	VUS	ND	ND	2	10	-	Y	N
72/F/24	p.G710S	Path	c.3400delC	VUS	57.7	96.6	0.6	12	448	Y	B
68/M/11	c.1708-1g>c	Likely Path	c.2866-3c>g	VUS	22.4	37.7	NA	6	-	Y	H
88/F/8	p.M769H-fs	Path	p.D1460Y	VUS	20.3	39.3	low	6	-	NP	H

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Table 2. Continued

Patient/ gender/age	Variant 1	Annotation 1	Variant 2	Annotation 2	ATP7B 1056 (pmol/L)	ATP7B 887 pmol/L	CPL (mg/dL)	Leipzig score	Liver Copper (ug/g Tissue)	KF Ring	Presentation
Patients with two VUS's (n=7)											
86/F/68	p.E332K	VUS	p.D1047V	VUS	51.7	79.3	27	6	26	NP	B
3/M/18	p.G1335E	VUS	p.G1335E	VUS	ND	ND	<2	-	-	YP	N
9/F/15	p.G1341E	VUS	p.G1341E	VUS	2.2	ND	1.4	6	-	NP	H
84/M/NA	p.I1336V	VUS	p.C709T	VUS	10.5	15.5	10	8	552	NP	H
85/F/NA	p.I1336V	VUS	p.C709T	VUS	ND	6.3	13	7	900	NP	H
186/M/NA	p.S932L	VUS	p.V1364V-fs	VUS	ND	ND	10	9	-	NP	B
130/M/20	p.T59H-fs*19	VUS	p.H1247Q	VUS	23.6	27.2	<4	9	54	Y	N
Patients with only one variant found (no 2nd variant detected) (n=18)											
208/M/17	c.2299delC	VUS	Unknown	-	ND	ND	<2	-	-	NP	H
209/M/19	c.2299delC	VUS	Unknown	-	ND	ND	<2	-	502	NP	N
195/F/43	c.51+4a>t	Path	Unknown	-	25.6	37.6	4	8	-	Y	N
196/F/28	p.A1049A-fs	VUS	Unknown	-	2.2	9.1	3	7	-	Y	N
194/M/50	p.D765N	Path	Unknown	-	20.4	21.4	<4	8	-	Y	N
197/M/22	p.G1176R	Path	Unknown	-	3.6	7.3	3	5	-	NP	H
199/M/17	p.H1069Q	Path	Unknown	-	8.6	32.9	<10	5	-	NP	N
201/M/14	p.H1069Q	Path	Unknown	-	31.4	29.0	3.4	-	-	NP	N
198/F/NA	p.H1069Q	Path	Unknown	-	ND	5.0	12	6	-	NP	H
200/M/14	p.H1069Q	Path	Unknown	-	ND	6.7	13	4	-	Y	H
211/F/22	p.H1069Q	Path	Unknown	-	ND	7.9	2	5	-	NP	N
210/M/NA	p.L1305P	Path	Unknown	-	ND	6.0	NA	3	-	-	-
202/F/16	p.M769H-fs	Path	Unknown	-	ND	ND	13.8	6	525	Y	H
203/M/37	p.R1319X	Path	Unknown	-	3.0	5.2	<10	9	1042	Y	B
204/M/12	p.R778L	Path	Unknown	-	ND	9.5	<3.0	5	-	NP	H
205/M/25	p.T1220M	Likely Path	Unknown	-	67.7	77.0	<10	4	191	NP	B
207/F/18	p.W779X	Path	Unknown	-	38.0	65.6	15.3	6	258	NP	H
206/M/45	p.W779X	Path	Unknown	-	ND	ND	<-4	6	-	Y	H

*, cirrhosis; A, asymptomatic; B, both hepatic and neurologic; H, hepatic; N, neurologic; NA, not available; ND, not detected; NP, not present; VUS, variant of uncertain significance; Y, present.

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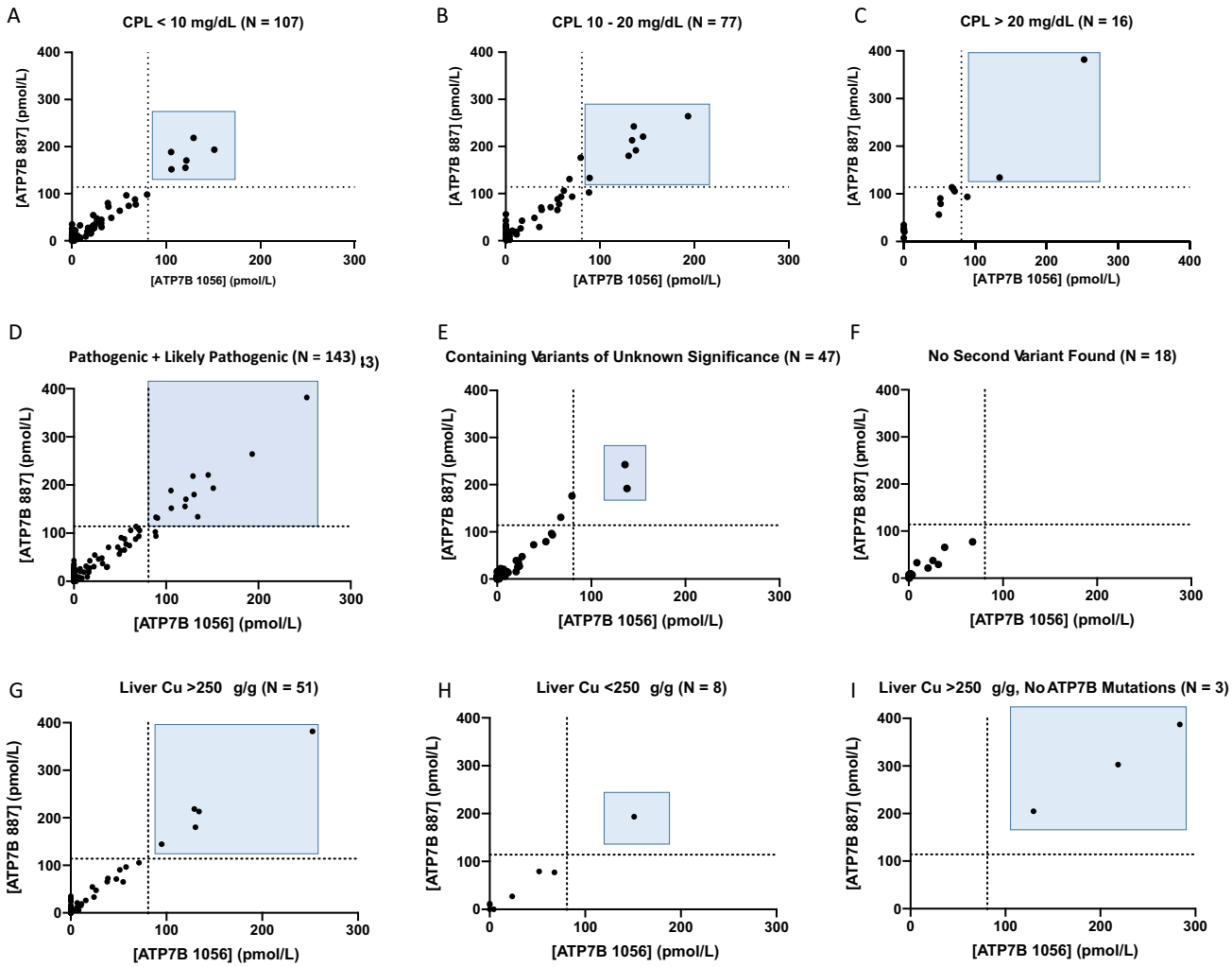


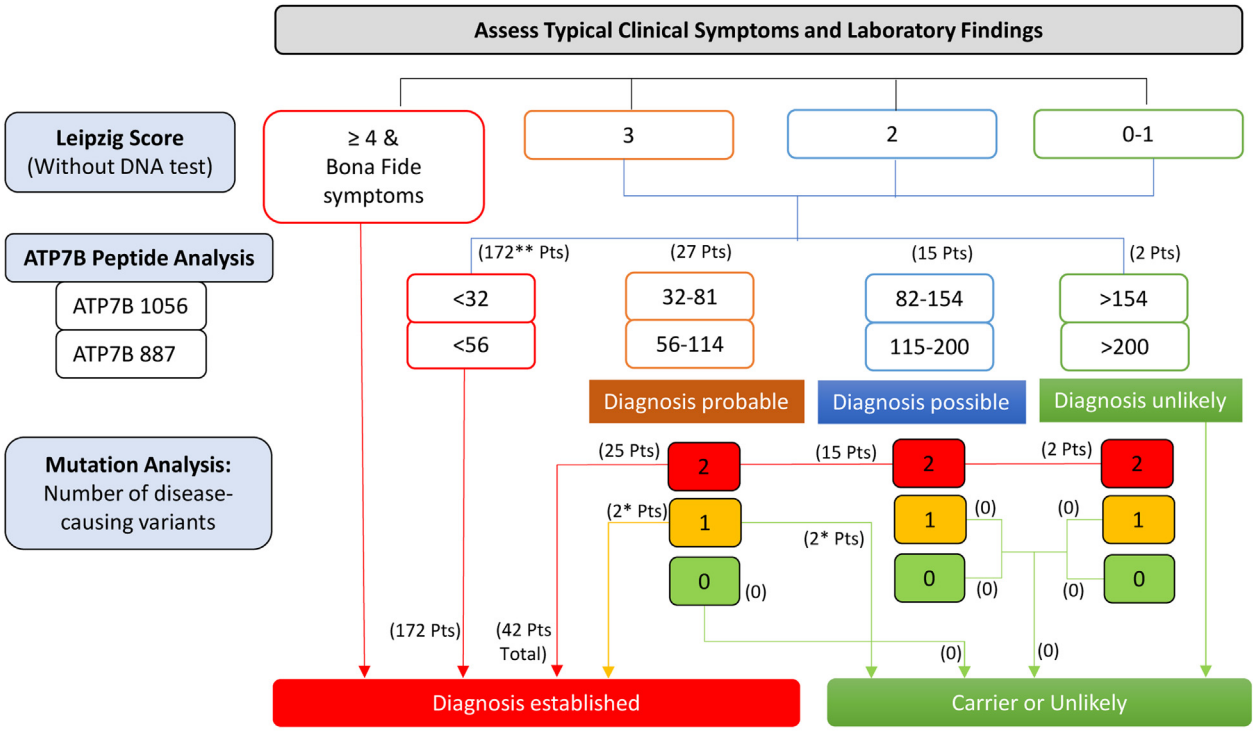
Figure 4. ATP7B peptide concentration analysis can provide clear results where Cp results and genetic analysis are ambiguous. Patients with significantly (*A*), moderately (*B*), and normal (*C*) Cp were readily identified. ATP7B concentrations are reduced regardless of variant status, including in patients with 2 pathogenic or likely pathogenic variants (*D*), at least 1 VUS (*E*), or where no second variant was found (*F*). *Dotted lines* represent peptide diagnostic cut-offs. Patients with liver copper above (*G*) or below 250 μg/g (*H*) are shown. In 3 samples from non-WD patients with elevated liver copper, ATP7B concentrations are normal (*I*).

for clinical laboratories.[26,27] The recommended Leipzig score for diagnosis of WD assigns numerical values to the number of disease-causing mutations to give probable WD diagnoses. The definition of "disease"-causing variant is based on a variety of databases, which can give conflicting answers.[28]

We hypothesized that direct measurement of ATP7B could identify WD patients as a majority of pathogenic mutations often result in protein misfolding, absence of decay of messenger RNA and enhanced degradation. To explore this goal, 2 WT ATP7B peptides were chosen for antipeptide antibody generation. Several factors influence peptide selection, as they must be unique to ATP7B, detectable by mass spectrometry, and elicit specific antibodies for isolation. ATP7B 1056 contains the most common WD-causing mutation, p.H1069Q. If the patient is homozygous for this mutation, WT ATP7B 1056 will not be found because the WT sequence is not present. Having a second peptide, ATP7B 887, builds a redundancy into the assay to ensure accurate performance.

As a primary diagnostic test, quantification of ATP7B from DBS effectively identified WD patients (Figure 2*A* and *B*, Table 1). Reduction of ATP7B concentrations below diagnostic cut-offs for at least 1 ATP7B peptide was evident in 92.1% of WD patients. Because the cut-offs set are based on the number of normal control patients analyzed, receiver operating characteristics curves were constructed showing ATP7B analysis to be highly sensitive and specific for diagnosis. The calculated area under the curve for the dataset is 0.98 regardless of peptide quantified (Figure 2*C* and *D*). Here, 211 patients had available genetic results (Figure 2*E*). WD is genetically confirmed in 143 patients with 2 evident pathogenic or likely pathogenic variants. This leaves 68 patients (32%) without a straightforward genetic diagnosis. Within the genetically confirmed subgroup, 91% were ATP7B-deficient, agreeing with sequencing results.

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*# 205 and #207 fall under both outputs: "Diagnosis established" and "Carrier or Unlikely".

**includes 156 patients with 2 variants and 16 patients with 1 variant

ATP7B	Average	SD	-3.5	-2.5	-2	-1
1056.0	203.0	48.9	31.9	80.8	105.2	154.1
887.0	257.7	57.5	56.5	114.0	142.7	200.2

Figure 5. Proposed Wilson disease diagnostic algorithm.

More importantly, 94% of patients without clear genetic results (containing VUS, VCI, or missing variants on sequencing) were ATP7B-deficient. ATP7B peptide concentration analysis can be highly useful in these patients.

ATP7B peptide concentrations were measured in patient samples collected up to 11 years prior, to study whether ATP7B degradation impacts stored samples (Supplementary Table 7). Individuals with reduced ATP7B in freshly or recently collected samples had reduced concentrations across time. This suggests that older samples are not being identified as patients due to ATP7B degradation. No diagnosis in this group changed due to date of sampling. This includes 1 normal control patient with 3 separate samples taken over 6 months. All measured concentrations clearly identified this individual as normal and the CV for the measurements in these samples was approximately 11.4% and 12.4% for ATP7B 1056 and 887, respectively.

Certain variants are highly prevalent in the population and are more commonly seen in clinic. These represent important test cases for the discriminatory ability of ATP7B quantification by immuno-SRM. These variants had predominantly low or undetectable peptide concentrations, supporting the hypothesis that protein levels are reduced in vivo. Four of these high-frequency variants (H1069Q, R778L, E1064A, and M645R) have significantly reduced ATP7B concentrations in both homozygotes and compound heterozygotes (Figure 3*A*–*D*). Patients with these common mutations and, therefore, a significant percentage of patients overall, should be readily discriminated by the use of immuno-SRM as an index test.

Direct measurement of ATP7B peptides means that disease-causing mutations that affect protein activity but not protein concentration will generate false-negative results. In each of the 17 false-negative patients, both ATP7B 887 and ATP7B 1056 were above diagnostic cut-offs, indicating significant production of nonfunctional protein. Identifying these variants and their frequency will be important in interpreting immuno-SRM in the context of other clinical results. If ATP7B concentrations in patient DBS are in the established normal ranges but clinical suspicion for WD is high, continued patient workup is obligatory to confirm the diagnosis. Several such variants were found here and ATP7B levels often depended on the nature of the second variant (Table 2). The variants found in these patients have AFs ranging from unknown to 0.0089% and would therefore represent a small percentage of the overall patient population.

The most common variants with ATP7B levels above the cut-off are p.G710S and p.R969Q (Table 2; Figure 3*E* and *F*). There were 13 WD patients carrying the p.G710S variant and 8 patients with p.R969Q. Seven p.G710S patients had normal concentrations of ATP7B peptides when they were

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either homozygous for p.G710S or compound heterozygous with p.G710A and p.M665I. Three p.R969Q patients were false negatives. In 2 of these cases, p.R969Q is in combination with p.H1069Q, which is shown to significantly reduce ATP7B concentrations. p.G710S is a variant associated with severe liver disease, 2 patients required an emergency liver transplantation due to fulminant hepatic failure.[29] These variants are known pathogenic by causing significantly impaired copper transport activity while maintaining normal ATP7B trafficking and phosphorylation in vitro.[24,30,31] Patients with these variants could be missed by immuno-SRM when in combination with variants producing significant ATP7B. When in combination with a variant severely affecting ATP7B concentrations, as in the p.H1069Q cases, ATP7B levels may give false negatives (Table 2). The mechanisms of variant interaction in vivo are currently under study. Diagnostic potential will therefore depend on the specific genotype of these patients. However, knowing that these variants have the potential to generate normal levels of ATP7B will be useful when evaluating genetic analysis after ATP7B measurement.

Like the method-dependent cut-offs used for Cp, 24-hour urinary copper[32] and hepatic copper for diagnosing WD,[33,34] measurement of ATP7B peptides require validation in the population in which it will be used. This will aid in generating clearly defined cut-offs. A true description of the final diagnostic performance of ATP7B concentration measurement will come with conducting a large cohort validation or pilot study, such as newborn screening.

Cp is not useful as a screening test for WD,[9] but levels of Cp <10 mg/dL are regarded as useful for diagnosis (score 2 in Leipzig score) of WD. A recent Chinese study found that Cp levels <12 mg/dL are strongly indicative of a diagnosis of WD.[32] Here, 200 patients had Cp values available, among them 77 (38.5%) had moderate Cp values of 10–20 mg/dL and an additional 16 (8%) had normal Cp levels >20 mg/dL; 92.9% of patients with Cp <10 mg/dL had ATP7B concentrations below diagnostic cut-offs (Figure 4*A*–*C*). When Cp levels were moderately reduced, 91.6% of patients would be identified by ATP7B analysis. Even in those with normal Cp values ATP7B identified 87.5% of cases. Measurement of DBS ATP7B can provide clarity when Cp levels are ambiguous. This is likely because ATP7B is not a measurement of secondary disease effects. Cp and copper measurements are often confounded by external processes or disease influences, including liver cirrhosis and malnutrition. As Cp is an acute-phase reactant possessing ferroxidase activity, the concentration can be elevated by acute inflammation. A prospective study on serum Cp as a screening test for WD in patients referred with liver disease reported a positive predictive value of only 6%.[9] Here, immuno-SRM analysis of ATP7B clearly outperforms Cp measurement.

Clinical information, including age, Leipzig scores, liver copper content, presence of KF rings, initial presentation, and presence of cirrhosis, was obtained when possible (Supplementary Table 3). No significant differences in ATP7B concentration were found based on age (pediatric vs adult), presence of KF ring, presentation (hepatic, neurologic, both hepatic and neurologic, or asymptomatic), or presence of cirrhosis. The mean ± SD ATP7B 887 peptide concentrations in hepatic and neurologic presentation were 33.8 ± 51.7 pmol/L and 38.2 ± 57.1 pmol/L, respectively. This appears aligned with previous observations in a large cohort showing the absence of any phenotype–genotype correlation regarding initial symptomatic manifestation.[35] Fifty-nine patients had hepatic copper measurements and 86.4% of these had elevated liver copper >250 μg/g. This includes 5 with normal ATP7B levels, 3 of them with p.G710S variant, and supports their diagnosis as WD patients despite negative immuno-SRM results (Figure 4*G*–*I*). Of interest, 3 samples were received with suspicion of WD by elevated liver copper but with no ATP7B variants identified. These samples had normal ATP7B concentrations, supporting their status as non-WD patients and providing an example of immuno-SRM analysis in ruling out WD (Figure 4*I*). Finally, it is unknown how many patients were being treated and what their treatment regimens were. Current WD therapies are focused on copper depletion and not restoration of ATP7B protein. As such, treatments would not affect the measured levels of ATP7B.

Examples From Dataset Where Diagnosis Is Simplified

The ability of ATP7B measurement to clarify case results extends to ambiguities in genetic analysis and contributes significantly to advancing noninvasive clinical diagnosis of WD (Figures 2*E* and 4*D*–*F*). There are 7 cases presented in which patients were compound heterozygous or homozygous for 2 VUS, indicating a lack of strong understanding of how these variants will contribute to phenotype (Table 2). All of these patients had significantly reduced ATP7B peptides levels suggesting affected patient status. Three patients in this group had only moderately reduced Cp between 10 and 20 mg/dL. In 1 case (no. 186), sequencing returned 1 VUS computationally predicted to be pathogenic and 1 (p.V1364V-fs) predicted to be benign. This patient had entirely nondetectable DBS ATP7B, indicating the consequence of this genetic combination is disease-causing. One patient (no. 86) presented an interesting clinical situation in which a VUS was found on sequencing and Cp concentrations were clearly within the normal range (27 mg/dL) (Table 2). Here, ATP7B measurement provided a clear indication that these patients severely lacked ATP7B protein and were very likely affected by WD in a way that genetic analysis, prediction, and Cp measurement could not.

Similarly, there were 37 patients in which a known pathogenic or likely pathogenic variant was found in combination with a VUS (Figure 4*E*, Table 2). Thirty-five (94.6%) of these had reduced ATP7B concentrations of at least 1 peptide, indicating positive identification as a WD patient despite a lack of knowledge of the consequences of their specific mutations. In 14 of these patients, Cp was found to be ≥10 mg/dL. Here, a significant reduction in patient ATP7B is particularly valuable for assigning a clinical designation and treatment course.

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Of concern are situations where Sanger sequencing and/or next-generation sequencing identifies only 1 variant. Sequencing analysis can be robust for targeting known variants but can return negative results if disease-causing mutations are small or large deletions, duplications, in the deep intronic or promoter regions, or in poly-A tails. These situations can have a significant impact on patient identification and place greater emphasis on Cp measurements and other WD diagnostics while the genetic basis for disease is not known. In 18 patients from our cohort with high suspicion for WD, only 1 *ATP7B* variant was identified (Figure 4*F*, Table 2). All of them had ATP7B measurement with peptide concentrations below established cut-offs. This includes 4 patients with Cp concentrations between 10 and 20 mg/dL, where a single variant and a moderate Cp reduction would be insufficient to establish the diagnosis. In 3 patients, the single variant detected from sequencing was a VUS causing a further complication in genetic interpretation. ATP7B measurement can provide direct evidence of the consequences of existing variants even if the second variant was not detected from sequencing workflows.

It is notable that some carriers with ATP7B peptide concentrations below cut-off create potential false positives. ATP7B 887 analysis showed 4 of 48 carriers had peptide levels below diagnostic cut-offs. The mechanisms by which a single affected allele reduce ATP7B concentrations are unknown and require study. Polymorphisms affecting protein concentration or factors affecting possible co-translated protein interactions may exist but are currently unknown.

Finally, we propose a new algorithm for WD diagnosis incorporating ATP7B measurement findings for each peptide (Figure 5). Here we define 4 possible patient groups based on ATP7B peptide level: directly established WD (<32 pmol/L for ATP7B 1056 and <56 pmol/L for ATP7B 887); probable WD (ATP7B between group 1 and the diagnostic cut-off); possible WD (ATP7B 1056: between cut-off and 154 pmol/L and ATP7B 887: between cut-off and 200 pmol/L); and unlikely WD (>154 pmol/L for ATP7B 1056 and >200 pmol/L for ATP7B 887). In validating the algorithm on the current retrospectively collected patients, we found that 172 of 216 (79.6%) showed ATP7B within the range of patient group 1, making their diagnosis very highly likely. Applying this method to the remaining cases along with mutation analysis show that all are able to reach WD diagnosis, except for 2 cases in which diagnosis or carrier status cannot be determined. Full validation of this proposed algorithm will be necessary through a large cohort prospective study.

Limitations

The study is limited in that data are predominantly for White patients. Patients from the Indian subcontinent, Africa, and South America may have different genotype distributions and possibly differing ATP7B peptide levels. There is also an unavoidable selection bias due to the analysis of only well-described WD cases. A significant limitation comes from the fact that these samples have been collected in a retrospective manner. It will be important to prospectively analyze a large cohort samples before genetic analysis in future applications. Full validation in a large prospective study will allow for more accurate definition of ATP7B variability in healthy normal samples and diagnostic parameters, including sensitivity, specificity, positive predictive value, and negative predictive value. The current statistical measures of predictive significance might change upon conducting a validating study. Furthermore, very few cases of fulminant WD and hemolysis were investigated. Patients presenting with hemolysis might have differing ATP7B peptide concentrations due to red blood cell lysis. In addition, ATP7B analysis is unable to delineate patient groups based on phenotype, that is, hepatic or neurologic types or disease severity. Finally, only 2 signature ATP7B peptides have been analyzed. Alternative peptide sequences may have different discriminating capability such that study of additional candidate peptides may be helpful.

LC-MS/MS is considered highly specialized technology, although it has been used in clinical laboratories in a wide array of fields, such as toxicology, drug monitoring, and newborn screening. Following the standard validation process in the clinical laboratory, we anticipate the assay can be successfully implemented into clinical practice.

ATP7B peptide analysis identified WD patients in a large majority of cases and reduced ambiguities resulting from genetic analysis and Cp levels. This noninvasive assay can serve as an adjunctive test for the diagnosis of WD and is expected to fundamentally advance the use of proteomic technology for a rapid screening tool, an area that holds great promise but is largely untapped.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dxdoi.org/10.1053/j.gastro.2021.02.052.

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Author names in bold designate shared co-first authorship.

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Correspondence
Address correspondence to: Peter Ferenci, MD, Medical University of Vienna, Vienna, Austria. e-mail: peter.ferenci@meduniwien.ac.at; and Si Houn Hahn, MD, PhD, University of Washington School of Medicine/Seattle Children's Hospital, Seattle, Washington. e-mail: sihahn@uw.edu.

Acknowledgments
Christopher J. Collins and Fan Yi contributed equally to this work.

CRediT Authorship Contributions
Christopher J. Collins, PhD (Data curation: Lead; Formal analysis: Lead; Writing – original draft: Equal).

Fan Yi, PhD (Data curation: Lead; Formal analysis: Lead; Methodology: Lead; Writing – original draft: Equal).

Remwilyn Dayuha, BS (Formal analysis: Supporting; Project administration: Supporting; Validation: Supporting; Writing – review & editing: Supporting).

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Phi Duong, BS (Formal analysis: Supporting; Project administration: Supporting; Validation: Supporting; Writing – original draft: Supporting).

Simon Horslen, MD (Resources: Supporting; Validation: Supporting; Writing – review & editing: Supporting).

Michelle Camarata, MD (Resources: Supporting; Validation: Supporting; Writing – review & editing: Supporting).

Ayse K. Coskun, MD (Project administration: Supporting; Resources: Supporting; Validation: Supporting).

Roderick H.J. Houwen, MD (Resources: Supporting; Validation: Supporting; Writing – review & editing: Supporting).

Tudor L. Pop, MD (Resources: Supporting; Validation: Supporting).

Heinz Zoller, MD (Resources: Supporting; Validation: Supporting; Writing – review & editing: Supporting).

Han-Wook Yoo, MD PhD (Resources: Supporting; Validation: Supporting).

Sung Won Jung, PhD (Data curation: Supporting; Formal analysis: Supporting).

Karl H. Weiss, MD (Resources: Equal; Validation: Supporting).

Michael L. Schilsky, MD (Resources: Lead; Validation: Equal; Writing – review & editing: Equal).

Peter Ferenci, MD (Data curation: Equal; Formal analysis: Equal; Resources: Lead; Supervision: Equal; Validation: Equal; Writing – review & editing: Lead).

Si Houn Hahn, MD, PhD (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Lead; Methodology: Equal; Resources: Supporting; Supervision: Lead; Validation: Lead; Writing – review & editing: Lead).

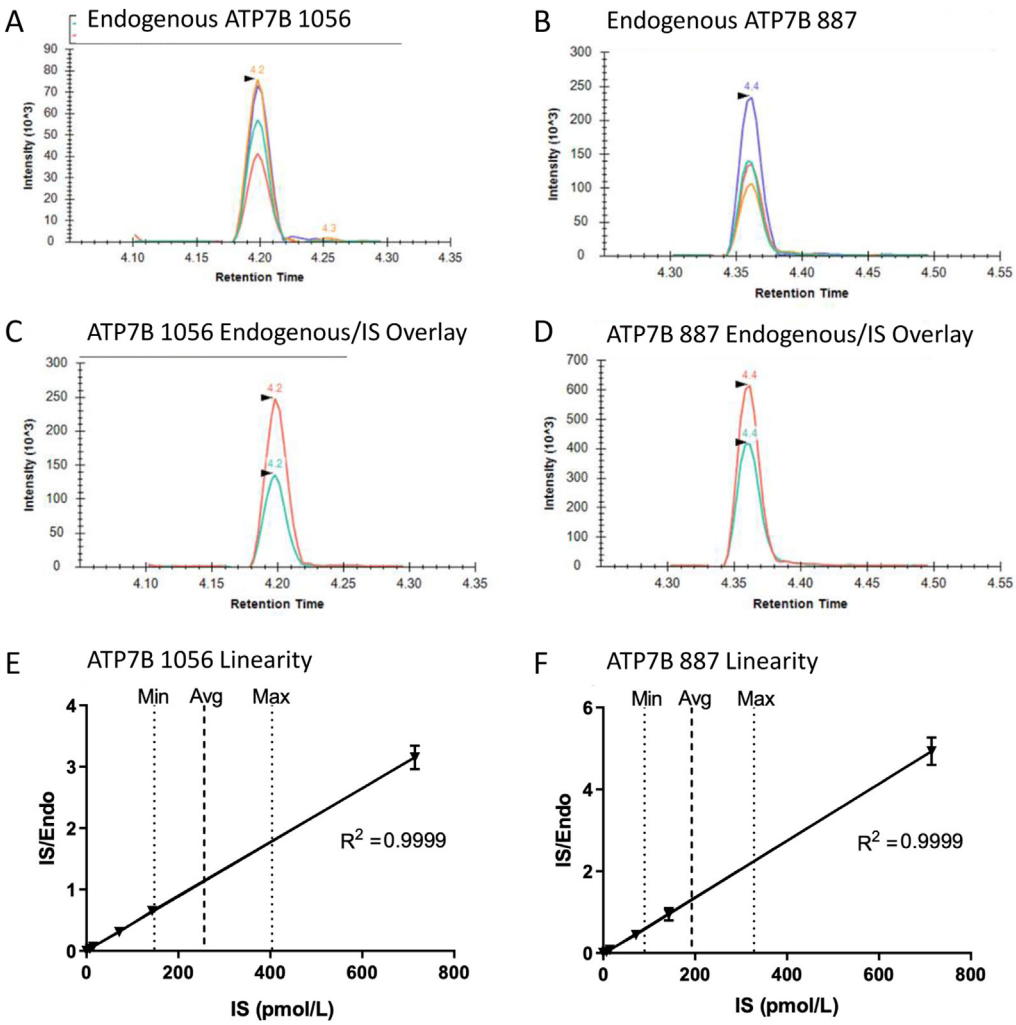
Conflicts of interest

The authors disclose no conflicts.

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Supplementary Figure 1. Representative chromatograms of ATP7B peptides in MS/MS. (*A*) endogenous ATP7B 887; (*B*) endogenous ATP7B 1056; (*C*) overlay of endogenous and IS ATP7B 887; and (*D*) overlay of endogenous and IS ATP7B 1056. Linearity curves of ATP7B 1056 (*A*) and ATP7B 887 (*B*). *Vertical lines* represent the average (avg), minimum (min), and maximum (max) concentration determined in normal controls.

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Correspondence
Address correspondence to: Arthur Kaser, Cambridge Institute of Therapeutic Immunology and Infectious Disease, Jeffrey Cheah Biomedical Centre, Puddicombe Way, Cambridge CB2 0AW, United Kingdom. e-mail: ak729@cam.ac.uk.

Conflicts of interest
The authors disclose no conflicts.

Most current article

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Expanding the Diagnostic Toolkit of Wilson Disease with ATP7B Peptides



See "Direct measurement of ATP7B peptides is highly effective in the diagnosis of Wilson disease," by Collins CJ, Yi F, Dayuha R, et al, on page 2367.

The diagnosis of Wilson disease (WD) can be determined by a combination of parameters aiming to detect copper accumulation. In this article from Collins et al,[1] a new approach to the diagnosis of WD is proposed. In current clinical practice, low ceruloplasmin levels are used as a minimal screening test, followed by Kayser–Fleischer ring assessment, 24-hour urine collection, liver biopsy for copper quantification, and brain magnetic resonance imaging for demonstration of copper-related changes in the basal ganglia. Although scoring systems can aid in the diagnostic process,[2] the traditional laboratory tools lack sensitivity and specificity for WD. In particular, the major limitation of ceruloplasmin determination is that most clinical laboratories quantify the enzyme levels, but not its oxidase activity, with consequent determination of erroneous elevated levels owing to quantification of both ceruloplasmin and biologically inactive apoceruloplasmin.[3]

Total serum copper is even less valuable as a screening test because it is influenced by ceruloplasmin level and its measurement methods. Genetic testing with *ATP7B* sequencing, whole-exome sequencing[4] and whole-genome sequencing are considered confirmatory tests and their costs are becoming more approachable. Although *ATP7B* variants are associated with various degrees of functional impairment,[5] their clinical correlate is uncertain. Therefore, the major challenge is the lack of genotype–phenotype correlation,[6] with the additional concern that some gene variants may not be associated with clinical manifestation development. Other diagnostic options, including the radioactive copper incorporation test[7] or the exchangeable copper,[8] are interesting but will likely offer several challenges in their access and execution outside selected academic centers. Therefore, although the diagnosis of WD is frequently achievable with available methods, many cases are trapped in a grey area of diagnostic uncertainty characterized by borderline ceruloplasmin levels, gene variants of unknown significance, and ambiguous clinical presentations. These borderline cases often overlap with common conditions, including fatty liver, autoimmune hepatitis, and movement disorders, and require refined clinical judgment and expertise for the final diagnosis.

Striving for a gold standard and improved diagnostic assessment is important because WD is a treatable disease both in the acute and chronic phases and timely diagnosis can prevent, improve, and even resolve many of the clinical manifestations. Particularly concerning are the neuropsychiatric manifestations, which are debilitating and only responsive after years of anticopper treatment and physical therapy. In addition, new treatment options are on the horizon, including next-generation chelating agents[9] and gene therapy approaches.[10] Medical treatment is often limited by drug toxicity, and unsatisfactory improvement, especially of the severe neuropsychiatric symptoms, is frequent. Liver transplantation can be an effective treatment and is often inevitable in cases of acute liver failure, but chronic immunosuppression is not desirable.[11] Therefore, when the phenotypic characterization of WD improves beyond the traditional definitions of prevalent hepatic and neuropsychiatric involvement,[12,13] the goal will be to tailor and optimize medical treatment to the phenotype or predicted disease course.

In this issue of *Gastroenterology*, Collins et al[1] adopt a new approach to the diagnosis of WD based on the quantification of ATP7B protein concentration derived from measurement of 2 surrogate peptides in patient dried blood

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spot samples, as direct evidence of WD diagnosis. Two ATP7B peptides, ATP7B 887 and ATP7B 1056, were selected and measured with specific antibodies. The studied samples derived from pre-existing biorepositories originated from European, North American, and South Korean institutions providing samples from patients with WD (n = 216), obligate *ATP7B* variant carriers (n = 48), and healthy patients (n = 150). Patients with WD carried different *ATP7B* variants, mostly highly prevalent in the population of origin. The age range was 2 months to 73 years, which is important because it is now established that the diagnosis of WD should be considered at any age.

The approach was first to identify the analytical and diagnostic performance of the peptides and identify the diagnostic cutoffs. A large proportion of the studied patients had available genetic test results and 92.1% of the patients with genetically confirmed WD had ≥1 of the peptides below the established minimal cutoffs. Among the 67 patients in whom genetic tests could not establish diagnosis of WD, 63 (94%) had their diagnosis clarified by low peptide levels. Of note, ≤16% of individuals heterozygous for WD had peptide levels below the cutoff, but could also have been WD cases carrying unknown variants. ATP7B 887 analysis demonstrated a sensitivity of 91.2% and specificity of 98.1%. In line with the challenges related to varied WD presentation, a small but sizable percentage of genetically confirmed WD cases presented peptide levels above the cutoffs, therefore representing a border line zone of patients that will likely still be difficult to diagnose or rule out as WD.

Owing to the retrospective nature of the sample collection, available clinical data were not extensive and information about current medical treatments were not detailed. Regardless, the possibility of a new diagnostic test to add to the WD toolkit available to practitioners not familiar with WD, is appealing and ultimately beneficial to patients. This study and the significance of the ATP7B peptides is timely as it positions itself in the middle of an ongoing debate about the full penetrance of WD,[14] the role of extra hepatic ATP7B copper transporter on clinical manifestations,[15] and the significance of ATP7B variants on disease phenotype.

Genetic testing and the knowledge to interpret these data remain incomplete. This study on ATP7B peptides opens clinical and research opportunities aiming to correlate peptide levels with disease severity and phenotype on larger populations. As suggested by the authors, integrating peptide levels into a validated diagnostic scoring system which includes copper metabolism parameters and genetic testing should be a major research focus and could lead to improved phenotypic and prognostic patient characterization. Ultimately, the proposed approach may be helpful to diagnose both adult and pediatric cases of WD.

In summary, expanding the diagnostic toolkit of WD is a priority and this study offers an additional option that could have a role as an initial screening or confirmatory test and will ensure that fewer diagnoses are delayed or missed.

VALENTINA MEDICI
University of California Davis
Department of Internal Medicine
Division of Gastroenterology and Hepatology
Sacramento, California

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Correspondence
Address correspondence to: Valentina Medici, MD, FAASLD, University of California Davis, Department of Internal Medicine, Division of Gastroenterology and Hepatology, 4150 V St, PSSB Suite 3500, Sacramento, CA 95817. e-mail: vmedici@ucdavis.edu.

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Most current article

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COVID-19 Gastrointestinal Symptoms and Attenuation of the Immune Response to SARS-CoV-2



See "Intestinal host response to SARS-CoV-2 infection and COVID-19 outcomes in patients with gastrointestinal symptoms," by Livanos AE, Jha D, Cossarini F, et al, on page 2435.

As we eclipse year 1 of the severe acute respiratory syndrome novel coronavirus-2 (SARS-CoV-2) pandemic, our understanding of this virus is only beginning to unfold despite an exponentially growing body of literature. Early descriptions of the novel respiratory illness coronavirus disease 2019 (COVID-19) were quickly followed by reports of a multisystem disease.[1] Gastrointestinal (GI) symptoms are frequently reported in patients with COVID-19, which raised questions of gut infection and the possibility of fecal–oral transmission. Indeed, viral RNA was detected in stool of infected patients by quantitative reverse transcriptase polymerase chain reaction, which frequently remained positive beyond the duration of positivity of their nasopharyngeal samples.[2–4] Multiple groups have reported GI epithelial coexpression of the host receptor ACE2 and TMPRSS2, a protease enabling cell entry through cleavage of the eponymous spike protein.[5–8] In line with the susceptibility of the GI epithelium to SARS-CoV-2 entry, viral RNA by in situ hybridization[9] and viral nucleocapsid protein (NP) by immunofluorescence have been detected in infected patient intestinal biopsies,[2] whereas viral particles have been demonstrated by transmission electron microscopy.[9] Moreover, recent studies using ex vivo enteroid or organoid models convincingly demonstrated that GI epithelium is permissive to SARS-CoV-2 infection.[6,9–11] Notably, human intestinal enteroids inoculated with the virus or nasopharyngeal aspirates from infected patients showed intracellular NP and increasing viral titers over time, indicating productive viral replication within intestinal epithelium.[9–11] Enteroids also exhibited double-stranded RNA, a requisite intermediate produced during the replication of this positive-sense RNA virus.[11] What happens in vivo remains unclear owing to the inherent logistical challenges of studying large cohorts of patients infected with this novel pathogen. Beyond the possibility of GI infection, viral interactions with GI tract tissues and the immune system, and how this influences clinical outcomes in COVID-19 remain largely unknown.

In this issue of *Gastroenterology*, Livanos et al[12] attempt to correlate clinical observations in patients with COVID-19 with immunologic changes within the GI tract on histologic, transcriptomic, and proteomic levels. The authors analyzed endoscopic biopsy specimens from COVID-19–afflicted patients, finding no gross abnormalities when compared with matched controls.[12] Although they found angiotensin-converting enzyme 2 (ACE2) diffusely expressed throughout the GI tract, viral NP was found more prominently in the ileum versus duodenum in the limited number of specimens studied.[12] They attribute this finding to increased MUC2+ goblet cell prevalence in distal small intestine, which colocalized with NP immunostaining, and their transmission electron microscopy images showing viral particles present primarily in exit vesicles of goblet cells.[12] This finding may corroborate findings from bronchial air–liquid interface cultures demonstrating tropism for goblet cells,[13] but were nevertheless surprising given relatively low ACE2 expression in intestinal goblet cell clusters,[5] evidence of goblet cell depletion in an infected patient's colonic biopsies,[9] and data from human intestinal organoids demonstrating enterocytes were susceptible to infection whereas goblet cells were not.[6,11] Livanos et al[12] also find evidence of NP staining in a nongoblet crypt base cell population but do not discern this population as Paneth, stem cells, or another population.[12] This raises additional questions of cellular tropism and host entry determinants because enterocytes in the villus compartment express the highest levels of ACE2.

The authors also performed a mass cytometry immunophenotypic analysis of endoscopic biopsies and blood samples from these patients.[12] They found that specific dendritic cell populations were diminished in the lamina propria of infected patients, possibly supporting some alteration in antigen presentation.[12] Bulk RNA sequencing of these tissues found decreased expression of several inflammatory pathways in the lamina propria and a trend toward the up-regulation of antiviral pathway signatures within the epithelial compartment.[12]

Notably, Vero E6 cells inoculated with homogenized biopsy tissue obtained from patients did not show evidence of productive infection.[12] This finding may simply result from sampling or technical error, but it may also reflect the biology of SARS-CoV-2 in the gut. There remains a striking lack of evidence of infectious virion isolation from the gut,

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Management of Wilson Disease Diagnosed in Infancy: An Appraisal of Available Experience to Generate Discussion

*Pamela L. Valentino, †Eve A. Roberts, ‡Stacey Beer, ‡Tamir Miloh, §Ronen Arnon, ||Jennifer M. Vittorio, and ¶Michael L. Schilsky

ABSTRACT

Increased access to molecular genetic testing is changing the demographics for diagnosing inherited disorders and imposing new challenges for medical management. Wilson disease (WD), typically diagnosed in older children and adults, can now be detected in utero and in infants (children younger than 24 months, including neonates) via genetic testing. An evidence-based approach to management of these neonates and extremely young children, who are typically asymptomatic, has been hampered by lack of clinical experience. We present a case of an infantile diagnosis of WD, review available experience, and discuss current trends in antenatal genetic testing of parents and fetus that may lead to a very early diagnosis of WD. Based on physiological and nutritional considerations, we propose an algorithmic approach to management of infantile WD as a starting point for further discussion. Future collaboration amongst specialists is essential to identify evidence-based approaches and best practice for managing treatment of infants with genetically diagnosed WD.

Key Words: *ATP7B*, copper, genetics, screening, zinc salts

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What Is Known

- ATP7B molecular genetic testing can identify homozygotes or compound heterozygotes for pathogenic mutations associated with Wilson disease.
- Screening first-degree relatives of patients with Wilson disease is recommended to identify those affected with Wilson disease.
- Zinc therapy is effective in asymptomatic patients with Wilson disease, but few data are available about optimal treatment for infants with genetically diagnosed Wilson disease.

What Is New

- Testing infants born to parents who are known carriers of *ATP7B* pathogenic mutations can identify infantile Wilson disease.
- Breast milk and formula provide approximately >150% of estimated copper and zinc needs, because copper is critical for normal development.
- Treatment with zinc in infantile Wilson disease may prevent development of significant copper accumulation and end-organ damage; however, timing when to start treatment is uncertain.
- When general newborn screening for Wilson disease becomes feasible, the resultant change in Wilson disease demographics will enhance the need for a coherent approach to management of infants with genetically diagnosed Wilson disease.

Wilson disease (WD) displays toxic accumulation of copper in liver and brain due to dysfunctional or absent ATP7B, the Wilson ATPase, an intracellular copper-transporting P-type ATPase expressed mainly in hepatocytes. ATP7B facilitates production of holoceruloplasmin and, importantly, biliary excretion of copper (1). The average age at diagnosis reported in pediatric WD cohorts is 10 years (2–4). Children older than 24 months with WD, herein referred to as having ''infantile WD,'' are almost invariably asymptomatic (5,6).

With availability of affordable expanded carrier screening-panels, we can detect the carrier status for various genetic diseases in parents. When each parent carries a mutation in *ATP7B*, genetic testing of the infant can lead to an early diagnosis of WD. Alternatively, whole-exome sequencing or sequencing for *ATP7B* mutations independent of the parents' status can also detect WD.

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From the *Department of Pediatrics, Section of Gastroenterology and Hepatology, Yale University School of Medicine, New Haven, CT, the †Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, Ontario, Canada, the ‡Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Houston, TX, the §Department of Pediatrics, Division of Hepatology, Icahn School of Medicine at Mount Sinai, the ||Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Columbia University, New York, NY, and the ¶Departments of Medicine and Surgery, Section of Digestive Diseases and Transplantation and Immunology, Yale University School of Medicine, New Haven, CT.

Address correspondence and reprint requests to Pamela L. Valentino, MD, MSc, FRCPC, Pediatric Gastroenterology and Hepatology, Yale School of Medicine, PO Box 208064, New Haven, CT 06520 (e-mail: pamela.valentino@yale.edu).

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M.L.S. is an advisor for Vivet therapeutics, Alexion, DepYmed, Kadmon, GMPO, and an investigator on sponsored studies with Alexion and GMPO. T.M. consults, advises, and is on the speaker board for Alexion.

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According to the American College of Obstetricians and Gynecologists Committee on Genetics, criteria for inclusion of a disease in expanded carrier screening-panels include a carrier frequency of ≥1:100, a well-defined phenotype, and onset of disease in childhood (7). Carrier rates for 1 *ATP7B* mutation are estimated at 1:90, except in some isolated populations; a recent British study reported a much higher carrier rate (8). WD is clinically pleomorphic, but its phenotypes are well described: ranging from mild with only liver test abnormalities to severe with hepatic decompensation during childhood, including acute liver failure requiring liver transplantation (2,9). Neurological and psychiatric symptoms are less frequent in childhood (2). With early institution of treatment, disease progression can be prevented and the morbidity associated with neurologic and psychiatric WD can be avoided (10). Thus, WD fits American College of Obstetricians and Gynecologists recommendations for carrier screening inclusion criteria.

Here we present a case of infantile WD as a basis for addressing the implications of genetic diagnosis during infancy. We have collectively reviewed current experience with infantile WD and examined infants' diets for exposure to copper and zinc. Copper is required for normal growth and development in infancy but is potentially toxic in excess. We propose an algorithmic approach for surveillance and management of infantile WD. Our purpose is to stimulate discussion around this emerging issue of how to manage this unique population, and promote multicenter collaborations to gather requisite data.

CASE PRESENTATION

The patient's mother was a healthy 35-year-old woman of Italian-Greek descent who had difficulty with conception due to a uterine septum. Before surgical correction, her fertility evaluation included carrier screening-panel testing. A heterozygous mutation in *ATP7B*, c.3207C>A (p.His1069Gln), was identified, which leads to rapid degradation of ATP7B in the endoplasmic reticulum (11). Her partner, of Italian descent, underwent genetic testing which identified a different heterozygous *ATP7B* mutation: c.845delT (p.Leu282Profs*2) producing a truncated protein (12). These 2 individuals were not known to be consanguineous.

The patient was born following a healthy pregnancy and delivery at term. Birth weight was normal. Genetic testing for *ATP7B* was obtained in the infant at 42 days. Results available by 2 months old revealed that she was compound heterozygote for the 2 parental mutations. Knowledge of the parental genotypes excluded that these 2 mutations were cis, thus affecting both alleles encoding ATP7B. At the initial hepatology evaluation, she was 3 months old, exclusively formula-fed and growing well (weight 76th percentile, length 98th percentile). On examination, she was well-appearing, anicteric, without hepatosplenomegaly. A routine unrestricted pediatric diet was recommended; foods with high copper content were not yet in the diet.

By 8 months of age, the infant was taking 24 oz of formula per day, equating to 329 μg of copper and 3.3 mg of zinc per day. She was ingesting a spectrum of infant purees, all in moderation, and demonstrated appropriate growth.

The infant was evaluated at 12, 15, and 18 months and was consistently asymptomatic with normal development, appropriate growth, and no hepatosplenomegaly. Laboratory results at these visits are included in Table 1. Abdominal ultrasonography at 12 months demonstrated a normal-appearing liver without increased echogenicity or hepatosplenomegaly (Fig. 1).

The parents' utmost concern had been the optimal strategy to avoid any copper overload in their child. At 18 months, she was clinically well and was eating a full diet of table foods, apart from foods high in copper (liver, nuts, mushrooms, chocolate, shellfish). Following discussion of risks and benefits with her parents, at this time treatment was initiated with zinc gluconate 25 mg twice daily, spaced away from meals. Since she was iron deficient before zinc initiation, iron supplementation was administered with meals twice a day. Zinc therapy was well-tolerated. We have not measured basal 24-hour urinary copper (or zinc) excretion as she was not yet toilet trained.

Cases of Infantile Wilson Disease in the Literature

Fourteen cases of infantile WD have been reported plus 2 unpublished cases (J.M.V., R.A.) (Table 2) (4,6,13–21). Genetic testing of infants born to children of known carriers of *ATP7B* mutations will identify patients with WD who are asymptomatic with normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Our review of the WD literature revealed that in cases diagnosed during infancy ALT values ranged between normal (12 IU/L) and approximately 10 times the upper limit of normal (556 IU/L). Data are skimpy; however, around the age of 11 to 13 months some had elevated aminotransferases (Table 2). Medical management of these cases was highly variable or else not described.

Physiological Considerations: Copper Metabolism During Fetal Development

Fetal copper trafficking is complicated. Both *ATP7A*, which is defective in Menkes disease, and *ATP7B* encode transporters involved in copper homeostasis in the placenta. ATP7A, the Menkes ATPase, allows copper to be imported across the placenta to the fetus from the maternal circulation (22). ATP7B is also expressed in the placenta and is expected to allow efflux of copper across the placenta back to the maternal circulation (22). In the third trimester this efflux, however, becomes less important as the growing fetus has increased copper needs. In infants whose placental ATP7B is defective, it is unknown if the total body copper is

TABLE 1. Longitudinal serum biochemistry measurements in an infant with Wilson disease before initiation of treatment

Age at testing	8 mo	12 m	15 mo	18 mo
ALT, U/L (normal <33)	21	25	15	20
AST, U/L (normal <32)	54	40	42	39
GGT, U/L (normal <42)	18	14	12	14
Direct bilirubin, mg/dL (normal <0.4)	0.2	0.1	< 0.2	<0.2
Ceruloplasmin, mg/dL (normal > 20)	8	11	11	7
Serum copper, μg/dL (normal 20–70)	25	27	28	25

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase.

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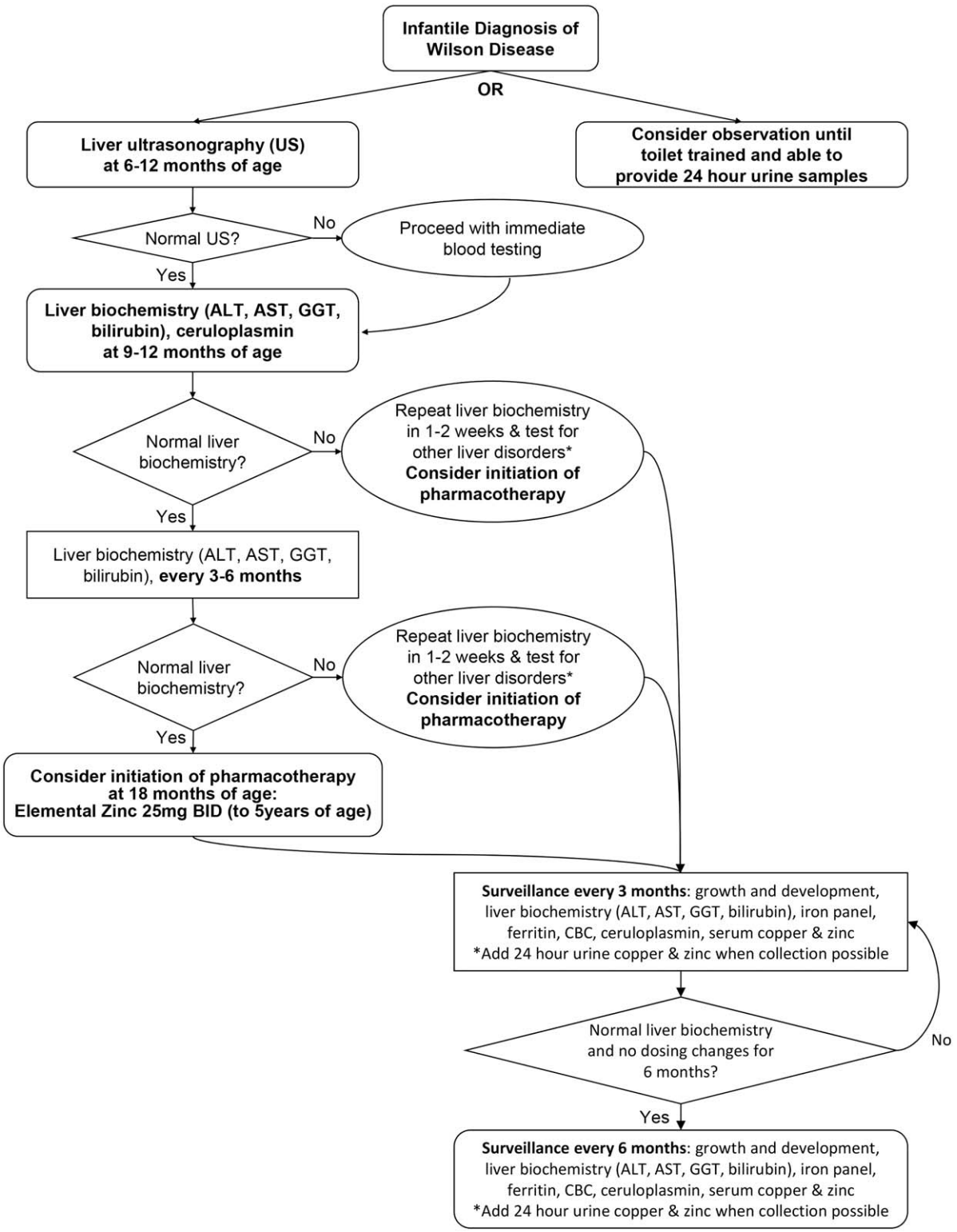


FIGURE 1. Proposed surveillance and treatment algorithm for infants with genetically diagnosed Wilson disease. *Etiologies of other liver disorders include alpha-1-antitrypsin deficiency, viral hepatitis B and C, and autoimmune liver diseases. ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase.

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TABLE 2. Reported and authors' experience with infantile Wilson disease

Presentation	Origin	ATP7B mutation	Ceruloplasmin, mg/dL	Urinary copper, μg/24 h	Aminotransferases at presentation	Management	Reference
Newborn boy; parents had prenatal genetic testing	USA	p.H1069Q/p.P1379S	26 (at 1 y)	220 (at 1 y)	ALT 110 IU/L AST 57 IU/L (at 1 y)	Low-dose zinc at 16 mo	Bennett et al, 2013 (13)
2-Mo-old girl; parents had prenatal genetic testing	USA (Italian/ Greek ethnicity)	p.H1069Q /c.845delT	8 (at 8 mo)	Not yet performed	ALT 21 IU/L AST 54 IU/L (at 8 mo)	Zinc started at 18 mo of age	Case presented here
3-Mo-old girl; parents had prenatal genetic testing	USA	c.4051C>T / c.2304dupC	<3 (at 6 mo)	Not yet performed	ALT 12 IU/L AST 23 IU/L (at 6 mo)	Dietary counseling	*J. Vittorio, unpublished observations
3-Y-old boy; parents had prenatal genetic testing	USA	p.H1069Q (homozygous)	26 (at 3 y)	23 (at 3 y)	ALT 30 IU/L AST 45 IU/L (at 3 y)	Zinc acetate started at 3 y	*R. Arnon, unpublished observations
4-Mo-old girl; family screening	Greek	Not available	Not available	<100	Not available	Not available	Manolaki et al, 2009 (4)
8-Mo-old boy; unclear reason for ceruloplasmin measurement	Japanese	c.2302insC (homozygous)	9.5 (at 8 mo)	17 (at 8 mo)	Within normal	Not reported	Shimizu et al, 1997 (14)
8-Mo-old boy; ALT measured with an acute diarrheal illness	Chinese	p.G1186S / c.4006delA, and 5 other single nucleotide polymorphisms	7.9 (at 11 mo)	Not reported	ALT 247 IU/L AST 193 IU/L (at 11 mo)	Zinc gluconate 10 mg TID started at 11 mo	Abuduxikuer et al, 2015 (6)
9-Mo-old boy; ALT measured with an acute diarrheal illness	South Korean	p.G1186S / c.4006delA	<9 (at 22 mo)	14.9 (at 23 mo)	ALT 122 IU/L AST 102 IU/L	Zinc 24 mg BID started at ~27 mo. Penicillamine initiated due to increasing aminotransferases	Kim et al, 2013 (15)
10-Mo-old girl; unclear why	Chinese	R776L/none found	Not reported	302	ALT 556 IU/L AST 274 IU/L	Trientine (dose and start date not reported)	Jang et al, 2010 (16)
11-Mo-old boy; family screening	Chinese	G943D/c.2299delC)	16	9 (0.14 μmol)	Not reported	Not reported	Mak et al, 2006 (17)
1-y-old; family screening	Lebanese	c.2299insC / c.2299insC	<2	10	ALT 44 IU/L AST 37 IU/L	Not reported	Usta et al, 2014 (18)
13-Mo-old girl; family screening	Italian	Molecular genetic testing did not reveal a mutation	8	4 (150 After penicillamine challenge)	ALT 210 IU/L AST 168 IU/L	Zinc sulfate ~23 mg BID started at ~16 mo	Iorio et al, 2003 (19)
13-Mo-old girl; unclear why	Italian	c.2299insG ho	7	Not reported	Not reported	Not reported	Nicastro et al, 2010 (20)
15-Mo-old girl; family screening	Chinese	L692P/L1015P	13.4	Not reported	Not reported	Not reported	Zhu et al, 2015 (21)
16-Mo-old girl; unclear why	Italian	P840L/N1270S	3	15	Not reported	Not reported	Nicastro et al, 2010 (20)
19-Mo-old boy; unclear why	Italian	c.2299insG ho	6	15	Not reported	Not reported	Nicastro et al, 2010 (20)
23-Mo-old girl; family screening	Greek	Not available	Not available	Not available	Not reported	Not reported	Manolaki et al, 2009 (4)

ALT = alanine aminotransferase; AST = aspartate aminotransferase.

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appropriate or if there is already an over-accumulation present at birth. The experience with the toxic milk mouse models of WD suggest that offspring of an affected dam, who are themselves homozygous for the same *ATP7B* mutation and destined to have murine ''WD,'' are relatively copper deficient: they do not survive unless foster-suckled by a normal mouse (23–25). Even less is known about the contribution to fetal copper homeostasis by a mother who is heterozygous for an *ATP7B* mutation; however, the normal circulating level of copper in these individuals suggests there should be no risk for copper deficiency in infants with breastfeeding. Further study is required to understand the copper balance at birth in infants with WD to guide nutritional and pharmacological recommendations.

With copper restriction or chelation therapy, copper deficiency can potentially arise. Infantile copper deficiency is rarely severe unless the child has Menkes disease. Copper deficiency may, however, develop in infants with malnourishment, premature birth, severe gastrointestinal disorders, intractable diarrhea, and parenteral/enteral nutrition dependence (26). Signs and symptoms of copper deficiency include anemia, neutropenia, bone or vascular lesions, and central nervous system disorders (26). Although copper deficiency is rare overall, the consequences to neurological development can be severe and irreversible (26).

Nutritional Considerations: Sources of Copper and Zinc in Infant Diet

Dietary counseling for patients with WD typically includes a recommendation to avoid foods with high copper content (27). Some pediatric practitioners attach little importance to these restrictions (28). Supplemental Table 1 (Supplemental Digital Content, *http://links.lww.com/MPG/B762*) includes a list of ''high'' copper foods, defined as copper content of >0.2 mg per portion. Foods that are most typically discussed in a restrictive diet are organ meat (liver), shellfish, mushrooms, chocolate, and nuts. These foods are not commonly consumed by infants; thus, their dietary exclusion is not a challenge. The concern for infants is the amount of copper contained in infant formula and baby foods, which tend to be heavy on copper (Supplemental Table 2, Supplemental Digital Content, *http://links.lww.com/MPG/B762*).

Another consideration is the amount of zinc in infants' diets, because dietary zinc may blunt the effect of copper intake by inhibiting gut absorption. The bioavailability of zinc in breast milk is higher than that in infant formulas (29). After birth, the concentration of zinc in breast milk is initially abundant, but by the age of 6 months, like other nutrients, it decreases quite rapidly, potentially putting an infant at risk for zinc deficiency (30). A vegetarian diet of plant-based foods, such as whole grains and legumes, contains less bioavailable zinc (29).

During the first 6 months of life the primary source of nutrition is almost exclusively breast milk or formula, which provides approximately >150% estimated copper and zinc needs. The more hydrolyzed protein formulas provide higher amounts of both copper and zinc. Copper and zinc content of these formulas must be balanced against the presence of gastrointestinal disease.

In infants 6 to 12 months old, breast milk and formula remain a primary source of nutrition, whereas complementary baby foods are added (Supplemental Table 2B, Supplemental Digital Content, *http://links.lww.com/MPG/B762*). Although many families continue to offer pureed baby foods, there has been a recent movement toward the practice of ''baby-led weaning'' where solid foods are offered in their whole form (31). Notably, many of the foods provided are high in copper (>>0.2 mg copper per portion [Supplemental Table 1, Supplemental Digital Content, *http://links.lww.com/MPG/B762*]), including beans/lentils, liver (as an alternative source of iron), avocado, and sweet potatoes.

In the second year of life, infants eat a more varied diet. An average balanced diet in children 12 to 24 months old, with the associated copper and zinc content, is presented in Supplemental Table 2C (Supplemental Digital Content, *http://links.lww.com/MPG/B762*). Many infants demonstrate picky eating behaviors, which may affect copper intake.

Management of Infantile Wilson Disease: Surveillance

Monitoring WD in infancy cannot simply be a modification of how we monitor adults. Infancy is a time of rapid growth and development and obtaining blood samples and timed urine collections is difficult. Reported clinical experience suggests that serum aminotransferases are seldom abnormal before the infant is 9 to 12 months old. This is a reasonable age at which to begin monitoring liver biochemistry: ALT, AST, gamma-glutamyl transferase, total and conjugated bilirubin, albumin, and serum ceruloplasmin. Liver biochemistry can be repeated every 3 to 6 months until starting pharmacotherapy. Detection of elevated liver biochemistry (>1.5 times the upper limit of normal) requires further investigation for other etiologies of liver disease including alpha-1-antitrypsin deficiency, viral hepatitis B and C, and autoimmune liver diseases. In the absence of other infantile liver disease, abnormal liver biochemistries may warrant initiation of therapy. The identification of an additional liver disorder may make it reasonable to delay commencing WD therapy until the effect of copper overload can be confirmed as the cause of elevated liver biochemistry. Such delays should not be unduly protracted.

Liver ultrasonography provides a noninvasive examination of the liver. It can provide pertinent information about hepatic steatosis (32). Obtaining a liver ultrasound around 6 to 12 months old in a healthy infant with genetically diagnosed WD, or earlier if symptomatic, may allay parental anxiety or provide a justification for earlier intervention.

The role of liver biopsy for surveillance is unclear. In general, it should be reserved for patients with liver test abnormalities in whom the diagnosis of WD is uncertain. The role of noninvasive surveillance markers, such as transient elastography, is indeterminate as few infants have been included in studies, and no studies have been reported for infants with WD.

Importantly, this strategy is also suitable for infants whose genetic testing reveals *ATP7B* alterations characterized as variants of unknown significance. Diagnosis in this situation is perplexing. Biochemical confirmation of copper overload is necessary in patients with genetic variants of unknown significance before starting life-long treatment; consultation with a geneticist may be indicated (27).

Management of Infantile Wilson Disease: Treatment

Little published data or experience are available to determine the optimal timing for starting pharmacotherapy in infants. We require data on copper status in this young population to help guide management. A 24-hour urine collection for copper and zinc quantification is not feasible in infants who are not yet toilet trained, unless a urinary catheter is placed (20). The risks of infection or trauma from 24-hour urinary catheterization are disproportionate. Because treatment monitoring is challenging during infancy, a conservative strategy may be most appropriate. The challenge is

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to balance achieving disease control against placing the infant at risk of copper deficiency during this critical phase of development.

Oral zinc is a well-established pharmacotherapy for the treatment of WD (10,33–36). Zinc, once absorbed, leads to increased synthesis of metallothionein (MT) in the enterocyte. MT binds copper preferentially over zinc, leading to sequestration of the MT-bound copper in the enterocyte and preventing copper uptake into the portal system (33). Copper is then removed via fecal excretion during regular enterocyte senescence. Zinc salts have fewer adverse effects than oral chelators; the main problem is adherence due to frequent dosing (ie, 2–3 times daily) spaced away from meals. The actual salt is not important for efficacy but may affect tolerability. In pediatric experience, zinc sulfate has been associated with notable gastric distress (33,37). A few zinc preparations exist and may theoretically differ in their tolerability and effect on appetite, nausea, vomiting, and diarrhea (38–40). Zinc as primary treatment for children with asymptomatic WD appears efficacious and well-tolerated (6,36,41,42) with the occasional dissenting report (43). Importantly, chronic treatment with zinc does not decrease hepatic parenchymal copper.

The dose of elemental zinc for treating young children (2–5 years old) is deduced from adult dosing regimens: 25 mg twice daily. This is a relatively high, truly pharmacological daily intake of zinc. Over the long term in adults with WD, zinc treatment may lead to actual copper deficiency, usually evident as anemia with neutropenia or leukopenia, although neurological deficits can develop. Little is known about any potential increased sensitivity of infants to the adverse effects of zinc treatment.

Thus, it seems reasonable to recommend zinc as initial therapy for infantile WD, based on available literature regarding management of presymptomatic children with WD diagnosed through first-degree relative screening (10). Admittedly, treatment options are limited since liquid preparations of chelators are not currently available. Based on review of previous pediatric WD case series, we would consider initial elemental zinc dosing of 25 mg per dose, twice daily, spaced 1 hour before, or 2 hours after meals, as suitable for the infant at the age of 18 months (34,44). If a suspension is unavailable, zinc should be dissolved in water. A more aggressive regimen would be smaller doses of elemental zinc (12.5 mg BID) starting at 12 months or earlier. Patients need to be monitored for copper deficiency with clinical examinations and complete blood count testing (45). Should evidence of copper deficiency develop, zinc can be held for approximately 2 to 3 months while monitoring neurological development and resolution of gastrointestinal symptoms or cytopenias. Further research is needed to determine the actual risk of copper deficiency with zinc treatment in infants with WD: it appears uncommon. Some infants may not benefit from zinc if they have advanced liver damage or persistently abnormal serum aminotransferases while on zinc therapy. They may actually require an oral chelator.

Another reasonable approach could be delaying treatment until liver biochemistry becomes abnormal or until the child is toilet-trained and can provide samples for 24-hour urinary copper measurement (46). Reported ages for successful toilet training vary in the literature, although many children are continent by 22 to 29 months.

Proposed Approach to Management of Infantile Wilson Disease: A Basis for Discussion

The broad outline of our proposal is confirmation of WD, preferably by molecular genetic testing identifying 2 trans mutations; close surveillance in the first 18 months of life; attention to dietary copper and zinc intake; initiation of zinc treatment at the age of 18 months; flexibility to alter this approach if the infant develops symptoms or biochemical evidence of liver disease earlier; and reassurance and support to parents throughout. The possibility that an infant could have WD plus some other more common liver disease cannot be disregarded. The difficulties of treatment monitoring need to be acknowledged upfront.

Special considerations relate to diet during infancy, warranting consultation with a dietician. Breast milk and baby formulas are surprisingly high in copper. Foods introduced at time of formula weaning are often high in copper. As the older infant transitions to an ordinary diet, exposure to most foods in moderation seems like a good plan. Parents may be fearful to allow their infant any intake of high copper foods, but relevant literature should be shared with parents to let them weigh risks and benefits of food choices. Addition of a chewable multivitamin (not containing copper) may be appropriate. Infants with WD should receive all routine vaccinations, including those against hepatitis A and B.

Arguably, given the variability of when WD becomes symptomatic, early treatment of asymptomatic, genetically diagnosed WD may be inappropriate. Further experience is required to address this issue. One possible strategy is that the nature of the mutation needs to be considered. Mutations predicted to result in absent ATP7B may be associated with earlier disease onset. The 3-year-old child presenting with cirrhosis due to WD was homozygous for a mutation predicted to interfere completely with production of the ATP7B (47). In a study of 59 genetically confirmed WD patients, those with 2 ''severe mutations'' (frameshift, nonsense, splice site mutations) had an earlier diagnosis of WD (median 13 years, interquartile range 9–13), whereas ''mild mutations'' were in patients diagnosed later (median 22 years, interquartile range 14–27) (48). An understanding of the infant's specific genetic mutations may influence when to start treatment, but this needs to be confirmed in formal study.

Future Directions

Family screening of first-degree relatives is already recognized as necessary following the identification of an index case. Infants born subsequent to such screening may be found to have WD (27). The scope of the problem of how to manage infants identified as having WD will increase substantially with the implementation of newborn screening. Early detection of WD via newborn screening may be feasible by quantifying ATP7B protein on dry blood cards (49). This approach is still in development.

Likewise, new treatments for WD may affect management of affected infants. Alternative therapies may include agents which ameliorate the effects of an ATP7B mutational folding defect (50). Recent findings show that inhibition of p38 and JNK pathways rescues ATP7B protein with a H1069Q mutation (51). Gene therapy, mediated by the adeno-associated virus, may become an option to correct mutations (52). Infantile WD may be the optimal setting for utilizing some of these treatments to prevent future organ damage.

CONCLUSION

Few data are available in the current literature to guide management of infants diagnosed with WD. Monitored clinical practice and multicenter collaboration will allow for an analysis of aggregate data and help guide future best management. Increased awareness of this issue and critical discussion are needed. Given a

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coherent management strategy, newborn screening for WD becomes more practical. Early detection and effective management of WD hold promise of avoiding severe WD in children.

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Wilson Disease

Synonym: Hepatolenticular Degeneration

Karl Heinz Weiss, MD
Internal Medicine
Salem Medical Center
Heidelberg, Germany
Email: karlheinz.weiss@stadtmission-hd.de

Michael Schilsky, MD
Yale University School of Medicine
New Haven, Connecticut
Email: michael.schilsky@yale.edu

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Summary

Clinical characteristics. Wilson disease is a disorder of copper metabolism that, when untreated, can present with hepatic, neurologic, or psychiatric disturbances – or a combination of these – in individuals ages three years to older than 70 years. Manifestations in untreated individuals vary among and within families.

- Liver disease can include recurrent jaundice, simple acute self-limited hepatitis-like illness, autoimmune-type hepatitis, fulminant hepatic failure, or chronic liver disease.
- Neurologic presentations can include dysarthria, movement disorders (tremors, involuntary movements, chorea, choreoathetosis), dystonia (mask-like facies, rigidity, gait disturbance, pseudobulbar involvement), dysautonomia, seizures, sleep disorders, or insomnia.
- Psychiatric disturbances can include depression, bipolar disorder / bipolar spectrum disorder, neurotic behaviors, personality changes, or psychosis.
- Other multisystem involvement can include the eye (Kayser-Fleischer rings), hemolytic anemia, the kidneys, the endocrine glands, and the heart.

Diagnosis/testing. The diagnosis of Wilson disease is established in most instances by a combination of biochemical findings (low serum ceruloplasmin concentration, low serum concentration of total copper, and increased urinary copper excretion) and/or detection of biallelic pathogenic (or likely pathogenic) variants in *ATP7B* identified by molecular genetic testing, based on the diagnostic scoring system developed at the 8th International Meeting on Wilson Disease.

Management. *Treatment of manifestations:* Lifelong medical interventions to prevent/treat copper accumulation need to be instituted as soon as possible in all individuals with Wilson disease whether they are asymptomatic (i.e., individuals with biallelic *ATP7B* pathogenic variants who have no clinical manifestations or tissue damage related to Wilson disease), clinically asymptomatic (i.e., individuals with biallelic *ATP7B* pathogenic variants who have no clinical manifestations of Wilson disease, but have Wilson disease-related tissue damage), or symptomatic (i.e., individuals with clinical manifestations of Wilson disease and Wilson disease-related tissue damage), regardless of age and including pregnant women. As Wilson disease treatment decisions might be complex, the consultation of disease experts (primarily hepatologists and neurologists) or Wilson disease centers of excellence is advised. The first-line therapy is copper chelating agents (D-penicillamine and trientine). Zinc salts (which interfere with absorption of copper from the gastrointestinal tract) cannot be used with a copper chelating agent and are most effective after initial decoppering with a chelating agent; however, in some individuals zinc salts can be used as an initial treatment. Orthotopic liver transplantation is used for individuals who fail to respond to medical therapy or present with fulminant acute liver failure.

The goals of supportive treatment for extrahepatic manifestations of individuals with symptomatic Wilson disease are individualized to maximize function and reduce complications. Depending on their clinical manifestations, symptomatic individuals may require specialists in neurology, occupational therapy, physical therapy, physiatry, orthopedics, nutrition, speech-language pathology, social work, and psychology/psychiatry.

Surveillance: To assess treatment effectiveness and adherence to medical interventions that prevent/treat copper accumulation, the following are recommended:

Feedback

- At least twice annually: assessment of serum copper and ceruloplasmin levels, liver biochemistries, international normalized ratio, complete blood count, urinalysis, and physical examination including neurologic assessment
- At least once annually: measurement of 24-hour urinary excretion of copper

Monitoring the individual's response to supportive treatment for extrahepatic manifestations and the emergence of new manifestations is per the recommendations of the treating clinical specialists.

Agents/circumstances to avoid: Foods very high in copper (liver, brain, chocolate, mushrooms, shellfish, and nuts) should be avoided, especially at the beginning of treatment.

In case of biochemical abnormalities in liver function tests or transaminases, alcohol consumption is strongly discouraged.

Evaluation of relatives at risk: It is appropriate to clarify the genetic status of asymptomatic older and younger at-risk relatives of an affected individual in order to identify as early as possible those who would benefit from prompt initiation of medical interventions to prevent/treat copper accumulation.

Pregnancy management: Treatment must be continued during pregnancy because of the risk for fulminant hepatic failure or irreversible neurologic deterioration. Because of possible adverse effects on the fetus from chelating agents, the dose should be kept as low as possible.

Genetic counseling. Wilson disease is inherited in an autosomal recessive manner. If both parents are known to be heterozygous for an *ATP7B* pathogenic variant, each sib of an affected individual has at conception a 25% chance of being affected, a 50% chance of being heterozygous, and a 25% chance of inheriting neither of the familial pathogenic variants. Once both *ATP7B* pathogenic variants have been identified in an affected family member, carrier and predictive genetic testing for at-risk relatives, prenatal testing for a pregnancy at increased risk, and preimplantation genetic testing for Wilson disease are possible.

Diagnosis

The diagnostic algorithm for Wilson disease in the European Association for Study of Liver (EASL) Clinical Practice Guidelines [European Association for Study of Liver 2012] is based on a diagnostic index ("Leipzig" score) proposed by an expert panel [Ferenci et al 2003]. This score includes clinical, biochemical, and molecular findings, but has not been validated in large patient series. The most recent diagnostic pathway of the American Association for Study of the Liver Diseases (AASLD) highlights diagnostic approaches when clinical and biochemical evaluations are ambiguous [Schilsky et al 2022b].

Suggestive Findings

Wilson disease **should be suspected** in individuals ages three to 45 years, but age alone should not exclude consideration of the diagnosis, as affected individuals have been diagnosed in their early 70s. At diagnosis, individuals with Wilson disease may have varying combinations of the following clinical findings, brain MRI findings (in those with neurologic manifestations), biochemical findings, and family history [Schilsky et al 2022b].

Clinical Findings

Children under age 18 years often present with hepatic disease exclusively.

Adults often present with hepatic disease with or without concurrent neuropsychiatric disease.

- **Liver disease** can range from recurrent jaundice, persistently elevated serum aminotransferase activity (AST, ALT), fatty liver, acute hepatitis (varying in severity, including acute liver injury), autoimmune-type hepatitis, and cirrhosis (compensated or decompensated) to acute liver failure (ALF).

  Note: Specific instances when Wilson disease should be considered is ALF with nonimmune hemolytic anemia or autoimmune hepatitis.
- **Neurologic manifestations,** resulting from central nervous system damage as a result of copper storage, can include the following:
  - Dysarthria
  - Movement disorders (tremors, involuntary movements, chorea, choreoathetosis)

- Dystonia (mask-like facies, rigidity, gait disturbance, pseudobulbar involvement)
- Dysautonomia
- Seizures
- Sleep disorders / insomnia

- **Psychiatric disturbances** can include depression, bipolar disorder / bipolar spectrum disorder, neurotic behaviors, personality changes, and psychosis.
- **Other extrahepatic involvement** can include the following:
  - Eye: Kayser-Fleisher rings, copper deposits in the periphery of the cornea, are observed by slit lamp examination and anterior segment optical coherence tomography (see Członkowska et al [2018], Figure 8). Sunflower cataracts and corneal nerve alterations can also occur.
  - Self-limited hemolytic anemia, with or without acute liver failure
  - Kidney abnormalities: aminoaciduria and nephrolithiasis
  - Hypoparathyroidism, pancreatitis
  - Cardiomyopathy, arrhythmias
  - Premature osteoporosis and arthritis
  - Infertility, recurrent miscarriages

Brain Imaging

Modalities such as magnetic resonance imaging (MRI) are of limited value in determining the extent of clinical neurologic disease but may help initially in supporting a diagnosis of Wilson disease and excluding other neurologic disorders.

Brain MRI findings consistent with Wilson disease include signal changes in the basal ganglia, thalami, pons, and white matter, as well as atrophy. Although the "face of the giant panda" sign (see Schilsky et al [2022b], Figure 1; full text), which consists of increased T_2 signal in the midbrain, has been considered pathognomonic for Wilson disease, several other findings are more commonly seen.

Biochemical Findings

Suggestive biochemical findings in a symptomatic individual relies on a combination of the following findings:

- Low serum ceruloplasmin concentration
  - **In children,** interpretation of test results requires age correction or age-specific reference ranges.

    Note: Healthy newborns have low serum ceruloplasmin concentrations. The concentrations increase during the first six months of life and peak by age two to three years at a concentration that may exceed the healthy adult reference range.
  - **In adults** with Wilson disease, serum ceruloplasmin concentration is often below the normal range (<0.2 g/L) and typically very low (<0.1 g/L).

    Note: A normal serum ceruloplasmin concentration is found in at least 5% of individuals with Wilson disease with neurologic manifestations and up to 40% of individuals with hepatic findings [Steindl et al 1997]. Serum ceruloplasmin concentration is, therefore, not a reliable screening test for Wilson disease.
- **Low serum concentration of total copper.** Most individuals with Wilson disease have a subnormal serum copper concentration that is proportional to the serum ceruloplasmin concentration (as ceruloplasmin is the main copper transporter in blood). The copper bound to ceruloplasmin (i.e., ceruloplasmin-bound copper) is considered nontoxic.

Note: Serum copper is low in healthy newborns. The concentrations increase during the first six months of life and peak by age two to three years at a concentration that may exceed the healthy adult reference range.

- **High urinary copper.** Measurement of copper in three 24-hour urine collections, free from contamination by external sources of copper, is advised. The testing laboratory should be consulted regarding its trace element urine collection protocol prior to initiating urine specimen collection.
  - **Basal urinary copper excretion** (without the use of chelating agents) is almost invariably elevated above 40 µg or ~0.6 µmol/24 hours in most individuals with Wilson disease, and above 100 µg or ~1.6 µmol/24 hours in symptomatic individuals.
  - **A provocative test of urinary copper excretion** following oral administration of D-penicillamine has been validated only in pediatric cohorts, but has proven useful in some adults [Martins da Costa et al 1992]; however, levels in affected individuals can overlap with those of heterozygotes. Note: The use of a lower value for basal urinary copper excretion of 40 µg or ~0.6 µmol/24 hours increases diagnostic sensitivity and may obviate the need for the D-penicillamine provocation test.
- **Hepatic copper quantification.** Although liver biopsy is an invasive procedure, it can be helpful when clinical findings, biochemical findings, and/or molecular genetic test results are ambiguous. Hepatic copper concentration in Wilson disease is usually greater than 250 µg/g dry weight (normal: <55 µg/g dry weight [Nuttall et al 2003]); however, such levels may be seen in other chronic liver disorders as well as cholestatic conditions [Schilsky et al 2022b].

  Note: (1) In later stages of Wilson disease, copper is distributed unevenly in the liver and measurement of hepatic copper concentration is less reliable. (2) Some individuals have only a moderately elevated hepatic copper concentration (100-250 µg/g dry weight), which overlaps with values occasionally found in heterozygotes. Thus, hepatic copper concentration in this range does not exclude the diagnosis of Wilson disease.

Family History

Family history is consistent with autosomal recessive inheritance. The family history may include affected sibs (e.g., sibs with liver disease, neurologic manifestations, and/or psychiatric disturbance) and/or parental consanguinity. Absence of a known family history does not preclude the diagnosis.

Establishing the Diagnosis

The diagnosis of Wilson disease, using clinical, biochemical, and molecular genetic findings, is based on the diagnostic scoring system developed at the 8th International Meeting on Wilson Disease, Leipzig 2001 [Ferenci et al 2003, Członkowska et al 2018] (see Table 1).

Table 1.

Diagnostic Scoring System for Wilson disease

Test	Parameter	Score
Typical clinical symptoms & signs		
Kayser-Fleischer rings	Present	2
	Absent	0
Neurologic manifestations [1]	Severe	2
	Mild	1
	Absent	0
Serum ceruloplasmin	Normal (>0.2 g/L)	0
	0.1-0.2 g/L	1
	<0.1 g/L	2

Test	Parameter	Score
Coombs-negative hemolytic anemia	Present	1
	Absent	0
Other tests		
Liver copper (in the absence of cholestasis)	>250 µg (>4 µmol)/g dry weight	2
	50-249 µg (0.8–4 µmol)/g dry weight	1
	Normal: <50 µg (<0.8 µmol)/g dry weight	-1
	Rhodanine-positive granules [2]	1
Urinary copper (in the absence of acute hepatitis)	Normal	0
	1-2x ULN	1
	>2x ULN	2
	Normal but >5x ULN after D-penicillamine	2
ATP7B molecular genetic testing	Biallelic pathogenic variants detected	4
	One pathogenic variant detected	1
	No pathogenic variants detected	0
Evaluation	**Total score**	
	Diagnosis established	≥4
	Diagnosis possible, more tests needed	3
	Diagnosis very unlikely	≤2

Adapted with permission from Ferenci et al [2003]

ULN = upper limit of normal

1. Or typical abnormalities on brain MRI
2. If no quantitative liver copper available

Per the diagnostic scoring system (see Table 1), the diagnosis of Wilson disease **can be established** in a proband with suggestive findings and biallelic pathogenic (or likely pathogenic) variants in *ATP7B* identified by molecular genetic testing (see Table 2).

Note: (1) Per ACMG/AMP variant interpretation guidelines, the terms "pathogenic variants" and "likely pathogenic variants" are synonymous in a clinical setting, meaning that both are considered diagnostic and both can be used for clinical decision making [Richards et al 2015]. Reference to "pathogenic variants" in this section is understood to include any likely pathogenic variants. (2) Identification of biallelic *ATP7B* variants of uncertain significance (or of one known *ATP7B* pathogenic variant and one *ATP7B* variant of uncertain significance) does not establish or rule out a diagnosis.

Molecular genetic testing approaches can include a combination of **gene-targeted testing** (single-gene testing, multigene panel) (see Option 1) and **comprehensive genomic testing** (exome sequencing, genome sequencing) (see Option 2). Gene-targeted testing requires that the clinician determine which gene(s) are likely involved, whereas genomic testing does not.

Option 1

Single-gene testing. When clinical and biochemical findings strongly suggest the diagnosis of Wilson disease, sequence analysis of *ATP7B* is performed first to detect small intragenic deletions/insertions and missense, nonsense, and splice site variants. Note: Depending on the sequencing method used, single-exon, multiexon, or whole-gene deletions/duplications may not be

detected. If only one or no variant is detected by the sequencing method used, the next step is to perform gene-targeted deletion/duplication analysis to detect exon and whole-gene deletions or duplications.

Note: Targeted analysis can be performed first in individuals from populations with known founder variants (e.g., Ashkenazi Jewish, Canary Islands, Druze, Sardinia; see Table 7).

A multigene panel that includes *ATP7B* and other genes of interest (see Differential Diagnosis) is most likely to identify the genetic cause of the condition while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype. Note: (1) The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time. (2) Some multigene panels may include genes not associated with the condition discussed in this *GeneReview*. (3) In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that includes genes specified by the clinician. (4) Methods used in a panel may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests.

For an introduction to multigene panels click here. More detailed information for clinicians ordering genetic tests can be found here.

Option 2

Comprehensive genomic testing does not require the clinician to determine which gene is likely involved. **Exome sequencing** is most commonly used; **genome sequencing** is also possible.

For an introduction to comprehensive genomic testing click here. More detailed information for clinicians ordering genomic testing can be found here.

Table 2.

Molecular Genetic Testing Used in Wilson Disease

Gene [1]	Method	Proportion of Probands with Pathogenic Variants [2] Detectable by Method
ATP7B	Sequence analysis [3]	98% [4]
	Gene-targeted deletion/duplication analysis [5]	Rare [6]

1. See Table A. Genes and Databases for chromosome locus and protein.
2. See Molecular Genetics for information on variants detected in this gene.
3. Sequence analysis detects variants that are benign, likely benign, of uncertain significance, likely pathogenic, or pathogenic. Variants may include small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected. For issues to consider in interpretation of sequence analysis results, click here.
4. Data derived from the subscription-based professional view of Human Gene Mutation Database [Stenson et al 2020]
5. Gene-targeted deletion/duplication analysis detects intragenic deletions or duplications. Methods used may include a range of techniques such as quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), and a gene-targeted microarray designed to detect single-exon deletions or duplications.
6. Large deletions and duplications, encompassing one or more exons, are rare. Exon and multiexon deletions have been reported (see, e.g., Møller et al [2005], Incollu et al [2011], Møller et al [2011], Tatsumi et al [2011]).

Clinical Characteristics

Clinical Description

Untreated symptomatic Wilson disease can manifest in individuals ages three years to older than 70 years as hepatic, neurologic, psychiatric, or hematologic disturbances, or a combination of these. Phenotypic expression varies even within families. The understanding of the phenotypic spectrum has further expanded through the widespread use of molecular genetic testing, which has confirmed the diagnosis in individuals with atypical clinical and biochemical findings.

Table 3 outlines the typical presenting clinical findings of untreated Wilson disease. Of note, the "classic triad" of liver disease, movement disorder, and Kayser-Fleischer ring is uncommon.

Table 3.

Clinical Findings in Individuals with Untreated Symptomatic Wilson Disease by Presenting Finding

Presenting Finding	% of Persons	Typical Age of Presentation (Range)	Liver Disease	Neurologic Disease	Psychiatric Disturbance	Kayser-Fleischer Rings
Liver disease	~40%	6-45 yrs (3-70 yrs)	+	+/–	+/–	~50%
Neurologic disease	~40%	Mid-teen to mid-adult (6-50 yrs)	–/mild	+	+/–	~90%
Psychiatric disturbance	~20%	Adolescent to young adult	–/mild	+/–	+	~90%
Hemolytic anemia	Few		+	–	–	+

Bruha et al [2011], Weiss et al [2011], Hofer et al [2012], Weiss et al [2013b]

Untreated Symptomatic Wilson Disease

Liver disease. Untreated Wilson disease manifests as liver disease more commonly in children and younger adults, typically between ages six and 45 years; however, severe liver disease can be the initial finding in preschool-aged children [Wilson et al 2000] and in older adults. The clinical manifestations vary and can include the following findings:

- **Recurrent jaundice,** possibly caused by hemolysis
- **Simple, acute, self-limited hepatitis-like illness** with fatigue, anorexia, and/or abdominal pain
- **Autoimmune hepatitis,** often manifesting acutely with fatigue, malaise, arthropathy, and rashes. This form of liver disease responds well to chelation therapy even if cirrhosis is present (see Management).
- **Fulminant hepatic failure** with severe coagulopathy, encephalopathy, acute Coombs-negative intravascular hemolysis, and often rapidly progressive renal failure. Serum activity of aminotransferases is only moderately increased, and serum concentration of alkaline phosphatase is normal or extremely low. These individuals do not respond to chelation treatment and require urgent liver transplantation (see Management).
- **Chronic liver disease** with portal hypertension, hepatosplenomegaly, ascites, low serum albumin concentration, and coagulopathy
- **Fatty liver** of mild-to-moderate degree with abnormal liver function

Neurologic involvement follows two general patterns: movement disorders or rigid dystonia.

- Movement disorders tend to occur earlier and include tremors, poor coordination, loss of fine motor control, micrographia (abnormally small, cramped handwriting), chorea, and/or choreoathetosis.
- Spastic dystonia disorders manifest as mask-like facies, rigidity, and gait disturbance [Svetel et al 2001].

Pseudobulbar involvement such as dysarthria, drooling, and difficulty swallowing is more common in older individuals, but also occurs in children and adolescents.

In contrast to the neurologic findings in individuals with a frank neurologic presentation, the neurologic findings in individuals with a hepatic presentation may be subtle. Mood disturbance (mainly depression; occasionally poor impulse control), changes in school performance, and/or difficulty with fine motor skills (especially handwriting) or gross motor skills may be observed.

In individuals with a neurologic presentation, extensive changes on brain imaging (such as evidence of tissue cavitation) suggest structural, irreversible brain damage. These individuals are less likely to improve with treatment [Sinha et al 2007].

Psychiatric manifestations are variable. Depression is common. Neurotic behavior includes phobias, compulsive behaviors, aggression, or antisocial behavior. Older individuals may have subtle psychopathology (e.g., progressive disorganization of personality with anxiety) and affective changes (e.g., labile mood and disinhibition). Pure psychotic disorders are uncommon.

Intellectual deterioration may also occur with poor memory, difficulty in abstract thinking, and shortened attention span.

Hemolytic anemia, with either acute or chronic hemolysis, indicates a high serum concentration of non-ceruloplasmin-bound copper, which leads to destruction of erythrocytes. Liver disease is likely to be present in such individuals, as are Kayser-Fleischer rings. Recurrent hemolysis predisposes to cholelithiasis, even in children.

Other extrahepatic involvement

- Kayser-Fleischer rings result from copper deposition in Descemet's membrane of the cornea and reflect a high degree of copper storage in the body. They do not affect vision and are reduced or disappear with effective decoppering treatment (see Management).
- Kidney involvement: low molecular weight proteinuria, microscopic hematuria, Fanconi syndrome, aminoaciduria, and nephrolithiasis
- Arthritis: involvement of large joints from synovial copper accumulation
- Reduced bone mineral density with an increased prevalence of osteoporosis (in approximately 10% of affected individuals)
- Pancreatitis, cardiomyopathy, cardiac arrhythmias, rhabdomyolysis of skeletal muscle, and various endocrine disorders
- Sunflower cataracts: observed occasionally on slit lamp examination

Hepatocellular carcinoma rarely develops in Wilson disease; the estimated incidence is below 1% [Devarbhavi et al 2012].

Fertility and pregnancy. Most individuals with Wilson disease are fertile.

Successful pregnancies of women with Wilson disease who received treatment have been reported [Brewer et al 2000, Tarnacka et al 2000, Furman et al 2001]. Prior to diagnosis and treatment of Wilson disease, affected women may experience amenorrhea, infertility, or recurrent miscarriage [Członkowska et al 2018].

Treated Wilson Disease

The mainstay of treatment for Wilson disease remains lifelong oral pharmacotherapy and dietary copper restriction [Schilsky et al 2022b] (see Management, Medical Interventions to Prevent/Treat Copper Accumulation). Liver transplantation, which corrects the underlying hepatic defect in Wilson disease, is reserved for individuals with chronic or acute liver failure and those resistant to pharmacotherapy.

- **"Asymptomatic individuals with Wilson disease"** are those who have biallelic *ATP7B* pathogenic variants who are clinically asymptomatic and **do not have any Wilson disease-related tissue damage**. Typically these individuals are young infants, born to parents known to be carriers, identified by genetic testing during family screening. These children should remain asymptomatic on treatment, even if they have biochemical abnormalities but not Wilson disease-related tissue damage. (See Management, Evaluation of Relatives at Risk and Medical Interventions to Prevent/Treat Copper Accumulation.)
- **"Clinically asymptomatic individuals with Wilson disease"** are those who have biallelic *ATP7B* pathogenic variants who are clinically asymptomatic but **have Wilson disease-related tissue damage**. Treatment at this stage of disease is highly successful and is focused on stabilizing and reversing tissue injury and preventing the progression of symptoms.
- **Individuals with Wilson disease with symptomatic liver disease.** Improvement in synthetic function and clinical signs such as jaundice and ascites begins during the first two to six months of treatment, with further recovery possible over time.
- **Individuals with Wilson disease with neurologic or psychiatric manifestations.** Most stabilize within six to 18 months after initiation of consistent therapy. However, neurologic findings may not respond to medical treatment, and in a few instances individuals with preexisting neurologic findings might show a paradoxical worsening, with acceleration of neurologic involvement or development of new manifestations.

Genotype-Phenotype Correlations

No genotype-phenotype correlations for *ATP7B* have been identified [Członkowska et al 2018, Ferenci et al 2019].

Nomenclature

The neurologic form of Wilson disease has also been known as Westphal-Strumpell pseudosclerosis.

Prevalence

The prevalence of Wilson disease is estimated at one in 30,000 in most populations, with a corresponding carrier frequency in the general population of one in 90 [Sandahl et al 2020].

In some population-based studies, the genetic prevalence was three to four times higher than clinically based estimates [Olivarez et al 2001, Coffey et al 2013], pointing to the complexity when classifying variants regarding its disease-causing potential and raising the question of whether penetrance is really 100%, as generally assumed.

Recent studies suggest a prevalence as high as one in 10,000, especially in isolated populations such as Sardinia [Gialluisi et al 2013].

Founder variants have been identified in persons of Ashkenazi Jewish and Druze heritage, as well as individuals from the Canary Islands and Sardinia (see Table 7).

Genetically Related Disorders

No phenotypes other than those discussed in this *GeneReview* are known to be associated with germline pathogenic variants in *ATP7B*.

Differential Diagnosis

The complete differential diagnosis of Wilson disease is extensive and includes:

- Copper metabolism disorders;
- Hereditary disorders involving the liver;
- Hereditary disorders involving the nervous system; and
- Acquired conditions such as viral hepatitis, severe drug toxicity, and nonalcoholic steatohepatitis (NASH).

Note: Wilson disease must be specifically excluded in individuals thought to have NASH, or the opportunity for life-saving treatment will be missed.

Table 4 lists selected genetic disorders of interest in the differential diagnosis of Wilson disease (see also Schilsky et al [2022b], Table 5).

Table 4.

Hereditary Disorders of Known Genetic Cause in the Differential Diagnosis of Wilson Disease

Gene(s)	Disorder	MOI	Copper Metabolism
Copper metabolism disorders			
AP1S1	MEDNIK syndrome (OMIM 609313)	AR	Low ceruloplasmin
ATP7A	Menkes disease (See *ATP7A*-Related Copper Transport Disorders.) [1]	XL	Low serum copper & low ceruloplasmin
	Occipital horn syndrome (See *ATP7A*-Related Copper Transport Disorders.)	XL	
	ATP7A-related distal motor neuropathy (See *ATP7A*-Related Copper Transport Disorders.)	XL	Normal
CP	Aceruloplasminemia [2]	AR	Low ceruloplasmin
SLC33A1	Huppke-Brendel syndrome [3]	AR	Low serum copper & low ceruloplasmin
Liver diseases [4]			
ABCB4	MDR3 deficiency (PFIC3) (See Pediatric Genetic Cholestatic Liver Disease Overview.)	AR	Hepatic copper retention due to cholestasis

Gene(s)	Disorder	MOI	Copper Metabolism
HFE	*HFE* hemochromatosis [5]	AR	Hepatic copper retention due to cholestasis is possible.
SERPINA1	Alpha-1 antitrypsin deficiency [5]	AD [6]	
Neurologic disorders			
ATN1	DRPLA	AD	Normal
DNAJC6 *FBXO7* *PARK7* *PINK1* *PRKN* *SYNJ1* *VPS13C*	Early-onset Parkinson disease (See Parkinson Disease Overview.)	AR	
GCH1 *TOR1A*	Inherited forms of dystonia incl DYT1 early-onset isolated dystonia & GTPCH1-deficient dopa-responsive dystonia	AD	
HTT	Huntington disease	AD	
NPC1 *NPC2*	Niemann-Pick disease type C	AR	
Many genes [7]	Hereditary ataxia	AD AR XL Mat [8]	

AD = autosomal dominant; AR = autosomal recessive; Mat = maternal; MOI = mode of inheritance; PFIC = progressive familial intrahepatic cholestasis; XL = X-linked

1. Onset during infancy
2. Iron overload due to lack of oxidase activity of ceruloplasmin
3. Characterized by cataract, sensorineural deafness, and severe developmental delay
4. Primary sclerosing cholangitis (OMIM 613806) and primary biliary cirrhosis (OMIM 109720) also present with abnormal liver biochemistries with or without hepatomegaly. The genetic basis of these disorders is unknown.
5. Presents with abnormal liver biochemistries with or without hepatomegaly
6. Alpha-1 antitrypsin deficiency is inherited in an autosomal codominant manner.
7. See Hereditary Ataxia Overview, Causes.
8. The mode of inheritance depends on the genetic etiology of ataxia.

Management

Evaluations Following Initial Diagnosis

To establish the extent of disease and needs in an individual with symptomatic untreated Wilson disease, the evaluations summarized in Table 5 (if not performed as part of the evaluation that led to the diagnosis) are recommended.

Table 5.

Recommended Evaluations Following Initial Diagnosis in Individuals with Symptomatic Untreated Wilson Disease

System/Concern		Evaluation	Comment
Primary manifestations			
Liver disease		Liver biopsy or biochemical testing & imaging of liver	• Establish baseline copper studies (serum ceruloplasmin & serum copper & 24-hr urinary copper excretion). • Consider additional upper GI endoscopy to exclude or confirm esophageal varices.
Neurologic		Neurologist assess for: • Movement disorders • Gait & balance disturbance	Using validated neurologic rating scale (neurologic subscale of Unified Wilson's Disease Rating Scale) [1]
Speech		For those w/dysarthria: eval by speech-language pathologist	
Musculoskeletal/ADL		Eval by physiatrist/OT/PT	To assess gross motor & fine motor skills, gait, ambulation, need for adaptive devices
Cognitive		Assess for cognitive dysfunction.	
Psychiatric		Eval by psychiatrist, psychologist, neuropsychologist if needed	For personality & mood disorders
Eyes		Complete eye exam	To incl assessment for Kayser-Fleischer rings, sunflower cataracts
Possible secondary manifestations			
Endocrine disorders	Glucose intolerance	Basic biochemical profile	Underlying liver disease might affect hormone metabolism.
	Parathyroid insufficiency		
	Disordered growth		
	Males: gynecomastia		
	Females: menstrual irregularity / amenorrhea		
	Frequent miscarriage		
Cardiac involvement	Cardiac arrhythmia	By cardiolologist	
	Cardiomyopathy		
Renal involvement		By nephrologist	Assess for: • Tubular dysfunction (e.g., aminoaciduria, hypercalcuria, hyperphosphaturia) • Nephrolithiasis, nephrocalcinosis
Genetic counseling		By genetics professionals [2]	To inform affected persons & their families re nature, MOI, & implications of Wilson disease to facilitate medical & personal decision making

System/Concern	Evaluation	Comment
Family support & resources	By treating clinicians, social workers	Assess need for: • Community or online resources; • Social work involvement for parental/caregiver support; • Home nursing referral.

ADL = activities of daily living; GI = gastrointestinal; OT = occupational therapist; PT = physical therapist

Adapted from Schilsky et al [2022b]

1. Członkowska et al [2007], Leinweber et al [2008]
2. Medical geneticist, certified genetic counselor, certified advanced genetic nurse

Treatment of Manifestations

Medical Interventions to Prevent/Treat Copper Accumulation in Individuals with Wilson Disease Who Are Asymptomatic, Clinically Asymptomatic, or Symptomatic

See extensive review by the American Association for the Study of Liver Diseases [Schilsky et al 2022b] (full text) and EASL Clinical Practice Guidelines: Wilson's disease [European Association for Study of Liver 2012] (full text).

Individuals with Wilson disease can be clinically categorized as:

- "Asymptomatic" (individuals who have no clinical manifestations or tissue damage related to Wilson disease);
- "Clinically asymptomatic" (individuals who have no clinical manifestations of Wilson disease but have Wilson disease-related tissue damage); or
- "Symptomatic" (individuals who have clinical manifestations of Wilson disease and Wilson disease-related tissue damage).

The goal of therapy is to institute treatment with chelating agents as soon as possible in individuals with Wilson disease who are asymptomatic, clinically asymptomatic, or symptomatic.

- Treatment is lifelong, including during pregnancy.
- If one treatment is discontinued, an alternative modality must be substituted to prevent disease progression.
- Discontinuation of all treatment leads to hepatic and neurologic decompensation that is usually refractory to further medical intervention.
- During lifelong treatment, failure of any medication used to treat Wilson disease may occur, either at initiation of treatment or during maintenance therapy. Once concurrent disease and nonadherence are excluded, pharmacologic therapy should be re-evaluated and likely altered. For individuals who have more advanced liver disease or develop liver failure, evaluation for liver transplantation should be considered. Currently, no surrogate markers are established for evaluating treatment failure.

Asymptomatic individuals should be treated either with lower dosages (10-15 mg/kg) of a copper chelating agent (D-penicillamine or trientine) or zinc salts.

Clinically asymptomatic individuals should be treated with 15-20 mg/kg of a copper chelating agent (D-penicillamine or trientine).

Symptomatic individuals should be treated with 15-20 mg/kg of a copper chelating agent (D-penicillamine or trientine). However, some individuals with advanced liver disease may require more intensive therapy, and temporally separated combination therapy may be utilized.

Copper chelating agents that increase urinary excretion of copper are the first-line treatment for persons with symptomatic Wilson disease. Note: Routine institution of chelation therapy before age three years has not been adequately assessed and may have adverse effects on growth.

- **D-penicillamine (chelator).** Used since the 1950s as first-line therapy for Wilson disease [Durand et al 2001, Walshe 2003], D-penicillamine is given as tablets by mouth two or three times daily. Pyridoxine must be given along with D-penicillamine. Twenty-four-hour urine copper excretion is used to confirm chelation and increased excretion of copper. Urinary copper values should be five to ten times normal; if the values are lower, noncompliance may be an issue, or body copper stores may have been adequately depleted.
    - Complete blood count and urinalysis must be monitored regularly during D-penicillamine therapy. Serious side effects can occur in up to 30% of individuals, and include severe thrombocytopenia, leukopenia, aplastic anemia, proteinuria, nephrotic syndrome, polyserositis, Goodpasture syndrome, and severe skin reactions. An early allergic reaction with fever, rash, and proteinuria may occur. Evidence of any such side effects may require discontinuation of D-penicillamine and substitution of an alternate treatment. If such alternate therapies are unavailable, D-penicillamine-induced adverse events may be manageable by coadministration of steroids.
    - D-penicillamine inhibits collagen cross-linking and has some immunosuppressant properties. After decades of treatment, individuals may have abnormal skin and connective tissue collagen, and possible chronic depletion of copper and (possibly) other trace metals.
    - D-penicillamine should NOT be used simultaneously with zinc, pending adequate clinical assessment of this treatment strategy.
- **Trientine (chelator),** also known as triethylene tetramine dihydrochloride (2,2,2-tetramine) or trien, has been the usual second-line treatment for individuals who cannot tolerate D-penicillamine. However, a clinical trial of an alternative formulation, triethylene tetramine tetrahydrochloride, revealed good efficacy and better tolerance than D-penicillamine, supporting the concept of its use as first-line therapy [Schilsky et al 2022a].
    - Complete blood count and urinalysis must be monitored regularly in all individuals on trientine.
    - Rare side effects include gastritis with nausea and, in cases of overtreatment, iron deficiency anemia.
    - Trientine should NOT be used simultaneously with zinc pending adequate assessment of this combination. Current reports suggest that the combination of trientine and zinc, temporally dispersed throughout the day such that each drug is administered five to six hours apart from the other, may be effective in severely decompensated hepatic Wilson disease [Santos Silva et al 1996, Askari et al 2003].

Zinc (metallothionein inducer). High-dose oral zinc interferes with absorption of copper from the gastrointestinal tract, presumably by inducing enterocyte metallothionein, which preferentially binds copper from the intestinal contents and is lost in the feces as enterocytes are shed in normal turnover. Zinc therapy is most effective after initial decoppering with a chelating agent [Brewer 2001, Brewer et al 2001]. In selected individuals, it can be used as an initial treatment [Milanino et al 1992, Linn et al 2009].

Zinc is taken as tablets by mouth at least twice (usually 3 times) daily before meals. The dose is based on the elemental zinc in the tablet.

Twenty-four-hour urine copper excretion is used to monitor total body copper stores, which should decrease. Increase of urinary copper excretion under zinc therapy may indicate insufficient treatment efficacy [Weiss et al 2011]. Serum or urinary zinc concentration can be measured to monitor compliance in individuals taking zinc.

Note: (1) Gastritis, a common side effect, can be reduced with the use of zinc acetate or zinc gluconate. (2) Zinc should NOT be used simultaneously with any chelator, pending further clinical investigation.

Restriction of foods very high in copper (liver, brain, chocolate, mushrooms, shellfish, and nuts) is likely prudent, especially at the beginning of treatment. It is recommended that individuals with special dietary needs (e.g., vegetarians) consult with a trained dietitian [Schilsky et al 2022b].

Orthotopic Liver Transplantation

Orthotopic liver transplantation (OLT) is reserved for individuals who fail to respond to medical therapy or cannot tolerate it because of serious adverse side effects [Schilsky et al 2022b].

It remains controversial whether orthotopic liver transplantation should be a primary treatment for individuals with Wilson disease who have severe neurologic disease [Medici et al 2005, Weiss et al 2013a, Litwin et al 2022].

Supportive Treatment for Extrahepatic Manifestations

The goals of supportive treatment for extrahepatic manifestations of individuals with symptomatic Wilson disease are individualized to maximize function and reduce complications. Ideally each individual consults with multidisciplinary specialists in fields such as neurology, occupational therapy, physical therapy, physiatry, orthopedics, nutrition, speech-language pathology, social work, and psychology/psychiatry, depending on the clinical manifestations.

Surveillance

Assessment of Treatment Effectiveness and Adherence to Medical Interventions to Prevent/Treat Copper Accumulation

Monitoring of individuals under therapy should include routine assessments of treatment efficacy by biochemical testing and clinical evaluation.

- Insufficient therapy, underdosage, or poor compliance could lead to reaccumulation of copper and development of new symptoms.
- Adverse events related to medical treatment (especially under D-penicillamine treatment) should be evaluated.
- Excessive long-term treatment could result in copper deficiency, leading to immobilization of iron (as observed in aceruloplasminemia) and neurologic symptoms of copper deficiency [Horvath et al 2010, da Silva-Júnior et al 2011].

According to current guidelines (AASLD [Schilsky et al 2022b] and EASL Clinical Practice Guidelines [European Association for Study of Liver 2012]), routine monitoring should include the following examinations:

- At least twice annually: serum copper and ceruloplasmin, liver biochemistries, international normalized ratio, complete blood count, urinalysis, and physical examination including neurologic assessment

  Note: Individuals receiving chelation therapy require a complete blood count and urinalysis regularly, no matter how long they have been on treatment.
- At least once annually: 24-hour urinary excretion of copper

  Note: Measurements are recommended more frequently if there are questions on compliance or if dosage of medications is adjusted.

Supportive Care

To monitor the individual's response to supportive care and the emergence of new manifestations, the evaluations in Table 6 are recommended based on the supportive treatment required by the individual.

Table 6.

Recommended Surveillance of Extrahepatic Manifestations for Individuals with Symptomatic Wilson Disease

System/Concern	Evaluation	Frequency
Neurologic	Assess for new manifestations such as seizures, changes in tone, & movement disorders.	At each visit
	Consider neuroimaging if new manifestations occur.	Per treating neurologist
Cognitive	Monitor educational needs.	At each visit
Speech & language	By speech-language pathologist & consideration of alternative means of communication	When clinically evident
Feeding	Eval of nutritional status & safety of oral intake	At each visit
Psychiatric/Behavioral	Behavioral assessment for depression, bipolar disorder, personality changes, & aggressive or self-injurious behavior	

System/Concern	Evaluation	Frequency
Musculoskeletal	Physical medicine, OT/PT assessment of mobility, self-help skills	
Family/Community	Assess family need for social work support (e.g., palliative/respite care, home nursing, other local resources), care coordination, or follow-up genetic counseling if new questions arise (e.g., family planning).	

OT = occupational therapy; PT = physical therapy

Agents/Circumstances to Avoid

Foods very high in copper (liver, brain, chocolate, mushrooms, shellfish, and nuts) should be avoided, especially at the beginning of treatment.

In case of biochemical abnormalities in liver function tests or transaminases, alcohol consumption is strongly discouraged.

Evaluation of Relatives at Risk

It is appropriate to clarify the genetic status of apparently asymptomatic older and younger at-risk relatives of an affected individual in order to identify as early as possible those who would benefit from prompt initiation of medical interventions to prevent/treat copper accumulation (see Medical Interventions to Prevent/Treat Copper Accumulation). Asymptomatic and clinically asymptomatic individuals with Wilson disease should remain asymptomatic on treatment, even if they have biochemical abnormalities, histologic findings, or imaging evidence of organ damage. Evaluations can include:

- Molecular genetic testing if the *ATP7B* pathogenic variants in the family are known;
- If the *ATP7B* pathogenic variants in an affected family member are not known, biochemical assessment of parameters of copper metabolism (serum copper, urinary copper, ceruloplasmin) and liver function tests as well as ultrasound imaging of the liver (the finding of a "fatty liver" is common, even in young or asymptomatic individuals) and slit lamp examination for the presence of Kayser-Fleischer rings.

  Note: Asymptomatic individuals with Wilson disease generally have a low serum concentration of ceruloplasmin and mildly increased basal 24-hour urinary copper excretion; however, sometimes asymptomatic individuals with Wilson disease cannot be easily distinguished from heterozygotes.

Although Wilson disease is an autosomal recessive disorder and the risk to the parents and offspring of a proband is low, screening of all first-degree relatives is recommended in order to ascertain clinically asymptomatic family members in whom treatment may prevent liver disease and other manifestations of Wilson disease [Schilsky et al 2022b].

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Pregnancy and Lactation Concerns

Pregnancy. Treatment must be continued during pregnancy because of the risk of fulminant hepatic failure and/or neurologic decline in the affected pregnant woman. Baseline biochemical and clinical assessment as soon as a pregnancy is recognized is important. This includes evaluation for portal hypertension in those who have cirrhosis because of the risk of peripartum variceal hemorrhage [Członkowska et al 2018].

- D-penicillamine has been used in many pregnancies with no adverse outcomes; however, congenital connective tissue disorders encompassing inguinal hernias and skin laxity have been reported in some exposed infants. Such adverse outcomes may depend on dose, which should be kept as low as possible while still preventing copper deficiency in the pregnant woman and accounting for the need for fetal copper during development [Członkowska et al 2018]. The dose of D-penicillamine should be maintained at the lowest effective dose during the first and second trimesters of pregnancy. Further reduction in dose may be considered in the third trimester – based on acceptable results of maternal biochemical liver function tests – to account for the increasing copper utilization by the growing fetus.
- Trientine has been used successfully during pregnancy, but the total number of reported individuals is small. Reduction of the dose to the lowest effective dose is recommended using a comparable approach to that for D-penicillamine.

- Zinc has been used effectively in pregnant women and typically does not require a decreased dose during pregnancy. However, changing medical therapy to zinc during pregnancy does not appear to decrease the risk of either miscarriage or adverse fetal outcomes [Członkowska et al 2018].

Lactation. All anti-copper medications appear to pass into breast milk, which can lead to copper deficiency in infants. Therefore, breastfeeding or using expressed maternal breast milk from a mother taking an anti-copper medication is not generally recommended [Członkowska et al 2018].

See MotherToBaby for further information on medication use during pregnancy and lactation.

Therapies Under Investigation

Tetrathiomolybdate (TTM) is an orally administered chelating agent proposed to work by multiple mechanisms [Plitz & Boyling 2019] including:

- Detoxifying non-ceruloplasmin-bound copper by creating a nonreactive tripartite complex with albumin and copper;
- Extracting copper from the endogenous cellular chelator metallothionein (based on its high affinity for copper); and
- Interfering with the intestinal uptake of copper when administered with food.

In a Phase II study [Weiss et al 2017], TTM effectively reduced non-ceruloplasmin-bound copper (corrected for copper-TTM-albumin complex) and improved clinical neurologic findings, without paradoxical neurologic worsening, as demonstrated by an overall improvement in Unified Wilson's Disease Rating Scale scores [Leinweber et al 2008]. Early elevation of serum aminotransferases in approximately 30% of individuals resolved with dose discontinuation or reduction; none developed evidence of drug-induced liver injury. Suitability for treating advanced hepatic Wilson disease requires further investigation. A Phase III trial of bis-choline TTM for Wilson disease is under way.

Search ClinicalTrials.gov in the US and EU Clinical Trials Register in Europe for access to information on clinical studies for a wide range of diseases and conditions.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, mode(s) of inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members; it is not meant to address all personal, cultural, or ethical issues that may arise or to substitute for consultation with a genetics professional. —ED.

Mode of Inheritance

Wilson disease is inherited in an autosomal recessive manner.

Risk to Family Members

Parents of a proband

- The parents of an affected individual are presumed to be heterozygous for an *ATP7B* pathogenic variant.
- If a molecular diagnosis has been established in the proband, genetic testing is recommended for the parents of a proband to confirm that both parents are heterozygous for an *ATP7B* pathogenic variant and to allow reliable recurrence risk assessment.
- If a pathogenic variant is detected in only one parent and parental identity testing has confirmed biological maternity and paternity, it is possible that one of the pathogenic variants identified in the proband occurred as a *de novo* event in the proband or as a postzygotic *de novo* event in a mosaic parent [Jónsson et al 2017]. If the proband appears to have homozygous pathogenic variants (i.e., the same two pathogenic variants), additional possibilities to consider include:
    - A single- or multiexon deletion in the proband that was not detected by sequence analysis and that resulted in the artifactual appearance of homozygosity;
    - Uniparental isodisomy for the parental chromosome with the pathogenic variant that resulted in homozygosity for the pathogenic variant in the proband.

- Clinical disease is not known to occur in heterozygotes (carriers), although the possibility has not been adequately excluded at older ages. Note: Heterozygotes may have subclinical biochemical findings including low serum ceruloplasmin concentrations, borderline normal urinary copper, elevated urinary copper on provocative testing with D-penicillamine, and/or moderate elevation of hepatic copper (100-250 mg/g dry weight).

Sibs of a proband

- If both parents are known to be heterozygous for an *ATP7B* pathogenic variant, each sib of an affected individual has at conception a 25% chance of being affected, a 50% chance of being heterozygous, and a 25% chance of inheriting neither of the familial pathogenic variants.
- Clinical symptoms may vary between sibs (including monozygotic twins) with untreated Wilson disease. The range of clinical variability observed between sibs with the same biallelic *ATP7B* pathogenic variants and treated Wilson disease depends primarily on the age of diagnosis and treatment initiation, reflecting the period of exposure to copper overload conditions.
- Clinical disease is not known to occur in heterozygotes (carriers), although the possibility has not been adequately excluded at older ages. Note: Heterozygotes may have subclinical biochemical findings including low serum ceruloplasmin concentrations, borderline normal urinary copper, elevated urinary copper on provocative testing with D-penicillamine, and/or moderate elevation of hepatic copper (100-250 mg/g dry weight).

Offspring of a proband

- Unless an affected individual's reproductive partner also has Wilson disease or is a carrier, offspring will be obligate heterozygotes (carriers) for a pathogenic variant in *ATP7B*.
- Given the carrier rate of one in 90 in the general population, the likelihood that an affected individual will have an affected child is one in 180. A higher carrier frequency is observed in some population groups due to founder variants (see Prevalence).
- Because the risk that an individual with Wilson disease will have an affected child is low, testing of serum ceruloplasmin concentration after age one year should be an adequate screening in offspring of a proband, except in populations with a high incidence of Wilson disease and/or a high incidence of consanguinity. In these populations, molecular testing may be useful. If molecular testing is not performed, repeat biochemical testing (including ceruloplasmin and urinary copper excretion) of offspring is strongly encouraged if initial biochemical testing was performed before age three years.

Other family members. Each sib of the proband's parents is at a 50% risk of being a carrier of an *ATP7B* pathogenic variant.

Carrier Detection

Molecular genetic carrier testing for at-risk relatives requires prior identification of the *ATP7B* pathogenic variants in the family.

Heterozygotes may have low serum ceruloplasmin concentrations, borderline normal urinary copper, elevated urinary copper on provocative testing with D-penicillamine, and/or moderate elevation of hepatic copper (100-250 mg/g dry weight), which make these tests unreliable in clarifying carrier status.

Related Genetic Counseling Issues

Predictive testing of adults and children. Because Wilson disease is a treatable condition, it is appropriate to offer predictive testing to asymptomatic at-risk adults and children (see Management, Evaluation of Relatives at Risk).

Family planning

- The optimal time for determination of genetic risk and discussion of the availability of prenatal/preimplantation genetic testing is before pregnancy.
- It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected, are carriers, or are at risk of being carriers.
- Carrier testing for the reproductive partners of affected individuals and known carriers should be considered, particularly if consanguinity is likely and/or if both partners are of the same ethnic background. Founder variants have been identified in some populations (see Table 7).

DNA banking. Because it is likely that testing methodology and our understanding of genes, pathogenic mechanisms, and diseases will improve in the future, consideration should be given to banking DNA from probands in whom a molecular diagnosis has not been confirmed (i.e., the causative pathogenic mechanism is unknown). For more information, see Huang et al [2022].

Prenatal Testing and Preimplantation Genetic Testing

Once the *ATP7B* pathogenic variants have been identified in an affected family member, prenatal and preimplantation genetic testing for Wilson disease are possible.

Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing. While most centers would consider use of prenatal testing to be a personal decision, discussion of these issues may be helpful.

Resources

GeneReviews staff has selected the following disease-specific and/or umbrella support organizations and/or registries for the benefit of individuals with this disorder and their families. GeneReviews is not responsible for the information provided by other organizations. For information on selection criteria, click here.

- **Association Bernard Pépin pour la Maladie de Wilson (ABPWilson)**
  France
  www.abpmaladiewilson.fr
- **Associazone Nazionale Malattida di Wilson**
  Italy
  www.malattiadiwilson.org
- **Deutsche Leberhilfe e.V.**
  Germany
  www.leberhilfe.org/lebererkrankungen/morbus-wilson
- **Morbus Wilson e.V.**
  Germany
  www.morbus-wilson.de/de
- **Wilson Disease Association**
  Phone: 866-961-0533; 414-961-0533
  Email: info@wilsonsdisease.org
  www.wilsonsdisease.org
- **Wilson's Disease Support Group - UK**
  United Kingdom
  www.wilsonsdisease.org.uk
- **American Liver Foundation**
  Phone: 800-465-4837 (HelpLine)
  www.liverfoundation.org
- **Canadian Liver Foundation**
  Canada
  Phone: 800-563-5483
  Email: clf@liver.ca
  www.liver.ca

- **Eurodis**
  Rare Disease Europe
  www.eurordis.org
- **Medline Plus**
  Wilson disease

Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A.

Wilson Disease: Genes and Databases

Gene	Chromosome Locus	Protein	Locus-Specific Databases	HGMD	ClinVar
ATP7B	13q14.3	Copper-transporting ATPase 2	ATP7B @ LOVD WilsonGen	ATP7B	ATP7B

Data are compiled from the following standard references: gene from HGNC; chromosome locus from OMIM; protein from UniProt. For a description of databases (Locus Specific, HGMD, ClinVar) to which links are provided, click here.

Table B.

OMIM Entries for Wilson Disease (View All in OMIM)

277900	WILSON DISEASE; WND
606882	ATPase, Cu(2+)-TRANSPORTING, BETA POLYPEPTIDE; ATP7B

Molecular Pathogenesis

The product of *ATP7B* is copper-transporting ATPase 2, an intracellular transmembrane copper transporter that is key in incorporating copper into ceruloplasmin and in moving copper out of the hepatocyte into bile. The protein is a P-type ATPase, characterized by cation channel and phosphorylation domains containing a highly conserved Asp-Lys-Thr-Gly-Thr (DKTGT) motif, in which the aspartate residue forms a phosphorylated intermediate during the transport cycle. The gene is expressed mainly in the liver and kidneys.

Tissue damage occurs after excessive copper accumulation resulting from lack of copper transport from the liver. Even when no transporter function is present, accumulation of copper occurs over several years.

Mechanism of disease causation. Various pathogenic variants lead to different impairments in *ATP7B* function.

***ATP7B*-specific laboratory technical considerations.** Comprehensive *ATP7B* testing should include promotor variants, as assessment of exonic sequences only does not rule out the diagnosis of Wilson disease when biochemical and/or clinical features are consistent with the diagnosis.

Table 7.

Notable *ATP7B* Pathogenic Variants

Reference Sequences	DNA Nucleotide Change	Predicted Protein Change	Comment [Reference]
NM_000053.4	c.-436_-422delTGGCCGAGACCGCGG	--	Founder variant common in Sardinia [Loudianos et al 1999]
NM_000053.4 NP_000044.2	c.1934T>G	p.Met645Arg	Founder variants comprising 85% of pathogenic variants in persons of Ashkenazi Jewish ancestry [Shi et al 2017]
	c.3191A>C	p.Glu1064Ala	
	c.3207C>A	p.His1069Gln	
	c.2123T>C	p.Leu708Pro	Founder variant common in Gran Canaria, Canary Islands, Spain [García-Villarreal et al 2000]
	c.3649_3654 delGTTCTG	p.Val1217_ Leu1218del	Founder variant in persons of Druze ancestry [Kalinsky et al 1998]

Variants listed in the table have been provided by the authors. *GeneReviews* staff have not independently verified the classification of variants.

GeneReviews follows the standard naming conventions of the Human Genome Variation Society (varnomen.hgvs.org). See Quick Reference for an explanation of nomenclature.

Chapter Notes

Author Notes

Dr Michael Schilsky's clinical and research interests include transplant hepatology, acute liver failure, and inherited metabolic disorders of the liver, in particular Wilson disease and hemochromatosis. Dr Schilsky co-wrote the AASLD and EASL practice guidelines for Wilson disease and chaired the writing group for the newly released 2022 AASLD practice guidance on Wilson disease. He is author of numerous original manuscripts and reviews on the subject. He is the Principal Investigator on clinical trials of pharmacotherapy and gene therapy for Wilson disease. Dr Schilsky is the organizer and Principal Investigator for the multicenter, multinational registry trial for Wilson disease sponsored by the Wilson Disease Association with data coordinating center at Yale University. He is a member of the NIH-sponsored Acute Liver Failure Study Group. He currently serves as Chair of the Medical Advisory Committee for the Wilson Disease Association.

Dr Karl Heinz Weiss's clinical and research interests include transplant hepatology, Wilson disease, and liver tumors. Dr Weiss co-wrote the 2022 AASLD practice guidance on Wilson disease. He is author of numerous original manuscripts and reviews on the subject.

Drs Weiss and Schilsky are actively involved in clinical research regarding individuals with Wilson disease. They would be happy to communicate with persons who have any questions regarding diagnosis of Wilson disease or other considerations.

Contact Drs Weiss and Schilsky to inquire about review of *ATP7B* variants of uncertain significance. Both authors are also interested in hearing from clinicians treating families affected by Wilson disease in whom no causative variant has been identified.

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Author History

Diane Cox, PhD, FCCMG, University of Alberta (1999-2013)
Eve Roberts, MD, FRCP(C), University of Toronto (1999-2013)
Michael Schilsky, MD (2023-present)
Karl Heinz Weiss, MD (2013-present)

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WILSON DISEASE ASSOCIATION
Unmasking Strength. Unleashing Promise.

July 23, 2024

Board of Directors
Washington State Department of Health

On behalf of the Wilson Disease Association (WDA), a patient organization dedicated to supporting and educating individuals affected by Wilson disease (WD), I am writing in support of your efforts to include Key Proteo's newborn screening test for newborn screening test (NBS) for Wilson disease in your state's screening panel.

WD is a genetic disorder that is fatal unless detected and treated before serious illness from copper toxicity develops. It affects approximately one in 30,000 people worldwide. The genetic defect causes excess dietary copper to accumulate in the liver or brain. Copper begins to accumulate immediately after birth. Over time, the excess copper harms the liver or brain, resulting in liver, neurologic or psychiatric symptoms. The symptoms usually appear in late adolescence to early adulthood but can occur at any age. WD is fatal if not diagnosed and treated.

Diagnosing WD is challenging for even the most experienced doctors since it can masquerade like many other disorders and is often misdiagnosed, sometimes for many years. Many tests are usually necessary, and sometimes the test results are inconclusive. Early diagnosis and proper treatment are essential to prevent progression of the disease.

We believe an effective newborn screening test would provide better outcomes for patients with Wilson disease so that treatment can be initiated as early as possible. The global Wilson disease community is very excited about the potential for NBS for WD.

We applaud your department for showing leadership in your efforts to have NBS for Wilson disease added to your state's screening panel. We hope that Washington state will be the first jurisdiction to adopt this test, setting the standard for other states to follow.

Thank you for all of your efforts in this area.

Best regards,

Rhonda Rowland

Rhonda Rowland
Vice President, Wilson Disease Association

224 W 35th St Ste 500 # 676 New York, NY 10001 866-961-0533 • 414-961-0533 •www.wilsonsdisease.org

Spanish Association of Relatives and Patients with Wilson Disease
Asociación Española de Familiares y Enfermos de Wilson
Molineta street, 1
04230 Huércal de Almería
Spain

To whom it may concern,

Wilson disease is caused by the accumulation of copper in organs such as the liver and brain. The longer it takes to diagnose and treat, the more copper will accumulate, increasing the probability of symptoms such as liver cirrhosis, speech and walking difficulties, psychiatric symptoms, and even death.

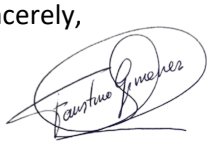
Early diagnosis and treatment of Wilson disease are essential for leading a normal life and avoiding serious complications. Many people remain undiagnosed and suffer from severe neurological symptoms or die from copper accumulation without ever knowing they had Wilson disease or receiving proper treatment.

Implementing newborn screening will improve the quality of life for the boys and girls diagnosed. It will lead to earlier detection, thereby enhancing our knowledge and treatment of the disease. This will also benefit those who are already diagnosed, providing them with more timely and effective care.

The Spanish Association of Relatives and Patients with Wilson Disease (Asociación Española de Familiares y Enfermos de Wilson) wholeheartedly supports the Wilson Disease Association and recognizes the critical importance of implementing newborn screening for Wilson disease in Washington. This initiative will serve as a model, encouraging its adoption in as many states and countries as possible worldwide.

Life has incalculable value, and newborn screening will save countless lives of those affected by Wilson disease.

Sincerely,



Faustino Gimenez Felices
President of
Spanish Association of Relatives and Patients with Wilson Disease
Asociación Española de Familiares y Enfermos de Wilson

UniversitätsKlinikum Heidelberg

krankenhaus salem
evang. stadtmission HD

Medical Clinic | Krankenhaus Salem | Zeppelinstr. 11-33 | 69121 Heidelberg | Germany

Academic teaching hospital
of Heidelberg University

Prof. (apl.) Dr. med. Karl Heinz Weiss
Chief Physician of Medical Clinic
Krankenhaus Salem

Zeppelinstraße 11-33 69121 Heidelberg
Phone +49 6221 483-201
Fax +49 6221 483-494
karlheinz.weiss@stadtmission-hd.de
www.krankenhaus-salem.de

WA State Department of Health

Board of Directors

Heidelberg, 20th July 2024

Dear Washington State Board of Health,

I am writing this letter in support of Dr. Hahn and Key Proteo, Inc. and their petition to include Wilson Disease (WD) on the list of conditions in the WA state newborn screening program.

As a physician and expert in Wilson disease, I have had the privilege of working with Dr. Sihoun Hahn, founder of Key Proteo, Inc., and can attest to their expertise in Wilson Disease and newborn screening.

Wilson Disease (WD) is an autosomal recessive disease of copper metabolism caused by pathogenic mutations in the ATP7B gene, a copper-transporting ATPase. About 1 out of 30,000 individuals are affected by WD. The symptoms typically appear between the ages of 5 and 45 years, and most affected individuals are clinically diagnosed often after they develop significant life-threatening complications, including liver cirrhosis, acute kidney failure, and brain and nerve damage. Early diagnosis and treatment of WD is highly effective in preventing these irreversible damages and can greatly improve the patients' quality of life. Currently there are no efficient and cost-effective screening methods available for early detection of WD. However, the technology developed by Dr. Hahn's laboratory and Key Proteo, Inc. allows early detection of WD prior to clinical manifestation.

Dr. Hahn has over 30 years of experience with WD and has been a pioneer in WD diagnosis. He found that WD patients have very low levels of ATP7B protein, making it a useful biomarker for WD screening. The Immuno-SRM technology developed at Key Proteo, Inc. allows highly precise quantification of ATP7B protein from dried blood spots and can successfully identify WD patients from healthy unaffected individuals. This assay can be readily performed by the WA newborn screening laboratory, since the laboratory already utilizes tandem mass spectrometry on the dried blood spot samples collected from newborn babies. Adopting Key Proteo's technology to screen newborns and detect WD before onset of clinical symptoms can prevent newborns from developing serious permanent complications that require long-term medical care, reduce the financial burden of WD treatment, and greatly improve the long-term clinical outcomes of affected patients.

I wholeheartedly believe that including WD in the WA state newborn screening program can make a lasting impact on thousands of newborns and their families. By testing newborns for WD and providing early intervention to those with WD, we can ensure these children are saved from life-threatening consequences and treated in a timely manner to enjoy healthy lives. Thank you for your consideration.

Sincerely,

Prof. Karl Heinz Weiss
MedicaL Director

Yale School of Medicine

MICHAEL L. SCHILSKY, M.D.
Professor of Medicine

Medical Director, Adult Liver Transplant
Yale-New Haven Transplantation Center

PO Box 208019 New Haven, CT 06510-8019
T 203-737-1592 F 203-785-6645
michael.schilsky@yale.edu

To:
WA State Department of Health
Board of Directors

From:
Michael L. Schilsky MD
Professor of Medicine and Surgery
Director, Center of Excellence for Wilson disease at Yale

Re: Newborn screening for Wilson disease

Dear Board members,

I am Professor of Medicine and Surgery and Director of the Wilson Disease Center of Excellence at Yale, and Chair of the Medical Advisory Committee for the Wilson Disease Association, and currently direct our clinical activities and research program on Wilson disease at Yale. I have over 35 years of experience in working in the field of copper metabolism and Wilson disease with clinical and research expertise in this area. I currently treat approximately over 320 patients with this disorder and direct the Wilson disease patient registry project and an NIH sponsored grant on biomarkers for copper metabolism.

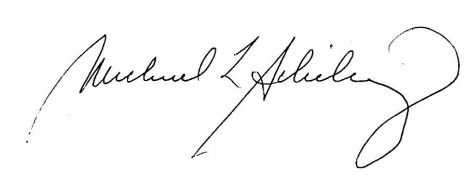
While we are fortunate to have treatments for Wilson disease, there are serious limitations of the available treatment options and disease caused disability due to the late diagnosis of this disorder. For these reasons, the work of Professor Hahn and his colleagues on identifying newborns with Wilson disease is so critically important. When the disorder is identified at an early stage before there is significant liver disease or extrahepatic disease, the patient can then be managed more easily with outcomes for survival comparable to those without the disease. Additionally, the medical and financial burden imposed by disease caused disability due to liver or neuropsychiatric disease can be avoided altogether. This is most desirable.

For these reasons I support the ongoing efforts to utilize the novel testing developed by Professor Hahn to detect this disorder in the earliest stages. We look forward to the successful implementation of the newborn screening program and to the challenge of working with younger patients to prevent disease throughout their lifetime, including the development of new therapeutics that may provide a cure for the disorder. However, all therapeutic efforts are dependent on the

ability to accurately detect the disease. Therefore, the incredible work that has been done by Dr. Hahn in advancing the diagnostics for Wilson disease to where it can be used in newborn screening is extremely important.

I most strongly support the ongoing work of Dr. Hahn and colleagues for the newborn screening project for Wilson disease, and very much appreciate your efforts to date. I look forward to the future universal use of this diagnostic test in clinical practice.

Sincerely,



Michael L. Schilsky MD FAASLD
Professor of Medicine and Surgery
Yale University Medical Center

July 18, 2024

Board of Directors
Washington State Department of Health

I am writing in support of Key Proteo and Dr. Sihoun's Hahn's newborn screening test for Wilson disease. I urge the Washington Department of Health to add Wilson disease to your state's newborn screening panel.

Please indulge me as I share my family's story with Wilson disease.

In December 2022 I had just retired from a 30+ year career in corporate communications and was looking forward to retirement. Our two children (son 23 and daughter 20) were successfully launched. My son had finished university and had a good job. My daughter was in her second year university studying economics and doing well. My husband and I congratulated ourselves on what a great job we had done raising our kids.

Then the walls caved in.

My daughter came home from university feeling very tired, bloated, nauseous and weak. I thought maybe she has irritable bowel syndrome, burnout from studying for exams, the freshman 15 weight gain, her appendix, gall bladder? I thought anything but Wilson disease.

Why would I? I have never heard of Wilson disease.

She suffered through the holidays and slept a ridiculous amount. The fluid was getting more pronounced in her abdomen and she started vomiting at random. A visit to our family doctor who took some blood work and an ultrasound showed fluid in her abdomen and elevated liver enzymes. "Let's monitor and test again in a month," our doctor advised. I realize now that if we had waited, she might not be here.

After the holiday break, my daughter insisted on going back to university to start winter semester. I suggested she stay home so we could arrange for more tests but she was eager to get back to school. She did agree that if the symptoms worsened or didn't improve, she would go to emergency. Two days later, she did just that. Little did we know that she was at end stage liver disease, due to Wilson disease, a condition that she had battled since birth, unbeknownst to any of us.

Fast forward a month later and she was back at home in Toronto Ontario with her new, transplanted liver.

To say we were blindsided is an understatement.

At emergency, doctors quickly diagnosed her with Wilson disease, within a few days. Her liver was so damaged that it couldn't be saved. She needed an emergency liver transplant.

I will never forget the doctor's words. "It's the only lifesaving option at this point." I was frozen and numb. How could this happen to an otherwise healthy young woman who didn't do drugs, didn't drink much, worked out and ate well?

We were lucky. My daughter was put at the top of the provincial transplant list in Ontario and received her new liver within 10 days of her Wilson disease diagnosis. I am forever grateful to her anonymous, deceased donor and their family who made the decision in their unimaginable grief to give back and do some- thing compassionate.

They saved my daughter's life.

While I am thankful for her new liver, I am still haunted with the knowledge that my daughter had a disease since birth that I had no idea about. If we had known that my daughter had Wilson disease as a baby, we could have potentially saved her from needing a liver transplant. She could have been monitored and treated so much earlier before the disease had progressed.

Sadly, my family's story is like many others. A difficult disease to diagnose because it can make other symptoms, people often endure significant liver, neurological and psychiatric symptoms, often irreversible. Left untreated, Wilson idea is fatal.

When I learned about Dr. Sihoun and Key Proteo's work with NBS for Wilson disease, I was very excited. It gives me great hope. If we could diagnose Wilson disease in infants, this could be a game changer and end so many years of suffering and anguish for patients and their families, not to mention significant health savings for the system.

Thank you for your consideration. I hope that Washington State can show leadership and courage and proudly stand as the first jurisdiction to add NBs for Wilson disease to its newborn screening panel.

Sincerely,

Alice Williams

Toronto, Ontario, Canada

To the Washington Board of Medicine

Our family's journey is one that is far too common with Wilson's Disease. Our son Rowdy was 17 years old and preparing to go serve an LDS mission for two years somewhere in the world. As a part of that process to get ready, he went in for his routine physical with our family doctor and friend. Two days after that physical I received an urgent phone call from our family doctor indicating that I needed to get Rowdy to the hospital lab to do additional work to figure out what was going on and that it was a mystery, urgently! As we were discussing things with the doctor, we learned that Rowdy's Platelet blood count was approximately 38 instead of the normal 200, also blood cell shapes and other things were mysteriously wrong. We did as requested and instructed by our doctor and urgently rushed him to the hospital lab to try to find out what was going on with his blood.

Over the course of the next six months with two different oncology specialist doctors we chased every leukemia, bone cancer, and blood cancer that are known. He underwent two separate bone marrow biopsies and was pulled from every physical activity that a normal 17-year-old boy does in fear of bleeding out and possible death. His world was turned upside down. Six months of tests, procedures, countless office visits to doctors and we had no answers. Following his second bone marrow biopsy, under the suggestion of two wonderful doctors at the Anchorage Providence Hospital, we started seeking an internalist to help coordinate all of the care for our son.

We reached out to our family doctor, and inquired who we should be seeing for internal medicine and he made some recommendations, one of the providers I knew. So we made our appointment and began yet another journey of unknowns, additional tests and more doctor visits. Our provider Karen Clements had remembered studying something about these types of mysteries while she was in school, and elected to do a larger blood panel to include many different enzymes and other such things in my son's body. When results came back, there were many red flags of concern as to the balance and health of my son's liver. A few more doctor visits, and our new provider had confirmed that our son had Wilson's Disease.

We had one answer and a whole New World of mysteries. We were referred to Biomedical Genetics at Seattle Primary Children's to begin coordinating care for my son. Additional tests were ordered again and more visits to doctors and hospitals. Ultrasounds, MRIs and CAT scans were ordered to deter,iome the extent of damage caused by the prolonged nature of undiagnosed Wilson's Disease. Our son, now 18-years-old has stage 4 cirrhosis of the liver that will never heal. The cirrhosis of the liver is a direct result of the disease and complications with the copper in his body. The required medication for an individual at this stage of Wilson's Disease is literally $40,000 a month without insurance. He will be on these medications for the next 3 to 5 years, possibly longer. We have now been working with Dr. Hahn and other specialists, geneticists, nutritionists and many others, in our wonderful care team, to treat my son's condition, to prolong the life of his liver, and give him the highest quality of life that he can have at this stage of cirrhosis.

We have a large family and as this is a genetic disorder, genetic tests were ordered for all of our children. This is how we discovered that his younger brother also has the disease. Teancum's journey will be very different from that of Rowdy because his disease was found 5 years earlier in his life. The cirrhosis in his liver is approximately 1.5 on the scale of 5. The

financial burden of the medication is not the same for him as he does not require the mining medication that Rowdy is on. He is able to treat his condition with diet and a therapeutic zinc taken daily, the cost of which is only about $40 a month. His life journey will be very different from Rowdy's because of an earlier diagnosis.

Due to the extent of the damage of Wilson's disease being undiagnosed in his body, there's a very high likelihood that Rowdy will require a liver transplant that could have been avoided with some form of early detection in place. Our family fully supports Dr. Hahn, his team, their research and the desire to make Wilson's Disease testing a functional and easy test at the time of birth. We already conduct many tests through a simple pin prick on the heel of every newborn child in every hospital across the United States to ensure the health safety and quality of life of those children. Wilson's Disease is uncommon and rare. It is debilitating and life altering, and a burden to be born by those who have it. If the State Board will embrace, endorse and approve the testing for newborn children in the hospital, the families of those children who test positive for Wilson's Disease will be able to begin their level of care at birth with low copper diets with high zinc intake. This prevents any damage, liver or the neurological damage, for those children, thus providing them a fullest possible life from beginning to end. Had this testing been available to our family, there's a high likelihood that Rowdy would not have level 4 cirrhosis. He would not be looking at the possibility of a liver transplant; he would be able to engage in regular activities as an 18-year-old young man, all of which is currently being denied to him because of the lack of early testing. If a simple pinprick could've changed my son's life. I would do it 1000 times over. Children should not have to worry about liver failure, Splenomegaly, and the possibility of bleeding to death because they play with their siblings.

A simple test could provide that peace to children. A test that they will never remember, a test that they will never have a memory of feeling,a test that they will never have the memory of conducting and changing their lives for the better. My family supports Dr. Hahn's proposal, and we would hope under the pleading of many many families who face this situation, the State Medical Board will also support this test and support his team in providing this to the newborn children. Once proven in Washington State, this test can be made available in other states, to other children, to other families. We talk about the potential elimination of diseases like polio, mumps, measles and rubella in the United States through simple testing and vaccines. Similarly the long-term debilitating effects of Wilson's Disease is virtually eliminated if care could begin on day one and patients would never feel the effects of the disease that they carry in their genetics, through no fault of their own. Please approve the institution of the heel prick testing of infants for Wilson's Disease.

Sincerely
Christopher and Rachel Johnson
Rowdy and Teancum Johnson
And family

Nora Closser
70 Barons Road
Rochester, NY 14617

July 25, 2024

Washington Department of Health
Board of Directors

To Whom it May Concern,

We are writing this in support of Dr. Sihoun Hahn's newborn screening assay for Wilson's Disease. Our story is like so many others that are diagnosed with a rare disease, it took a very long time to get a diagnosis. The caveat to Wilson's Disease is that unlike many other rare genetic diseases-it is treatable and in most cases manageable. Early diagnosis would have prevented so much disability and heartache that we have endured in the last 23 months of our lives. Our hope is that no family has to go through what we have gone through.

Our 13 year old daughter, Brinley started with abrupt muscle spasms one day at school in October, 2022. These muscle spasms and myoclonus progressed over the course of the next 2 months to balance issues, swallowing issues, extreme fatigue and weakness. I cannot describe the look of terror on her face when she could not control her own body. When we could not get in to see a neurologist or an outpatient MRI of her brain, our former pediatrician recommended taking her to the Emergency Department. The attending neurologist told us that he believed this was a functional disorder and that a brain MRI was not necessary unless we needed it to get on board because in order to treat a functional disorder-formerly known as a conversion disorder, all parties need to be on board. I have been a nurse for over 25 years and in pediatrics for the last 15. I told them we needed the MRI. It was unremarkable. They did not do any additional labs or testing at that 24 hour admission. She was placed on Gabapentin and continued the Hydroxyzine that she was taking. Two months later they added Amantadine because she couldn't focus. She could not focus because she could not control the myoclonus or tremors she was experiencing.

She missed her entire year of 6th grade.
We did home tutoring. The diagnosis of Functional/Conversion disorder followed her until diagnosis.

Her symptoms progressed. She started with severe leg pain in March of 2023. No medications helped her. In May of 2023, she started with severe back pain. Her former pediatrician told me that the only things that would help her would be to get her to sleep, get her to school, get her in a pool and refused to write a continuation letter for home tutoring. The school nurse knew my daughter well and let me call her into school every day. She knew that something was really wrong and urged me to go to a bigger city like Boston and present to their ER. She had been seen by Infectious Disease, Neurology, and Rheumatology. Symptoms progressed. She had

mottling of her hands, Mee's lines on her nails, and looked sickly. I vowed that I would find an answer for her. This was not my daughter. I knew something was very wrong. I researched for hours many nights trying to find an answer. Brinley researched with me. In May of 2023, we changed pediatricians. I had researched all of her new symptoms and Wilson's Disease came up as a differential diagnosis. Her liver function and brain MRI was normal. It wasn't on the radar because of these factors. I needed to talk to a provider who while she didn't know me on a personal level, knew me on a professional level and knew my clinical assessment skills. She immediately wrote the note to continue home tutoring. I asked about Wilson's Disease and she ordered a ceruloplasmin test, which had not been done on this journey. It was low. There was finally something that could explain all of her symptoms. I told Brinley that we may be on the right path to a diagnosis and she said, "Mom, I know this is what it is."

Referral to GI was the next step. She had suffered from unexplained abdominal pain since the age of 10 so we already had a relationship there. The pediatrician sent in the referral the evening the ceruloplasmin level was low. I messaged the GI provider the next morning and she ordered the initial liver screening work up. Everything was still normal except for the ceruloplasmin level. The 24 hour urine screen for copper was normal. We met with the liver team who ordered a referral to Genetics. All of this took time. The positive genetic test did not come back until August 2023. When I told Brinley about the positive test, her response was "I knew it."
No medications helped her symptoms. She tried and failed so many times during this course. She was immediately referred to a Pediatric Hepatologist at Columbia as there were none in our area. We had one in Rochester that left the area 2 days prior to the genetic test results. She was immediately started on Zinc and then did a Penicillamine challenge. She had a seizure with the first dose. When we presented to the ER, we were told by the attending Neurologist that it was likely functional. With one dose, she still had excessive copper in the 24 hour urine. We needed to quantify how much copper was present given the normal liver labs and MRI's. A liver biopsy confirmed high copper content. Columbia wanted us to see specialists at Yale as they are a Center of Excellence. We were seen by Dr. Bamfort at Yale in early December 2023 and then by Dr. Schilsky on December 22, 2024. She started Trientine on December 23, 2023.

Brinley missed her entire year of 7th grade at school.

As a result of her positive test, our 10 year old daughter, Emma was found to be positive for Wilson's disease. She is currently taking trientine. She is asymptomatic and will hopefully remain that way since she was started on treatment earlier. She is diligent about taking her medication and adhering to the dietary restrictions because she saw the hell her sister went through. She never wants to get to that point.

Family testing revealed my nephew was positive and his sisters were carriers.

Brinley took every medication they tried, did all tests ordered, every MRI, every lab draw, every 24 hour urine without a complaint. Never did she say "Why me?". She knew there was an answer, we needed to find it and was willing to do anything to get there.

The impact of a delayed diagnosis took its toll on every aspect of our lives. Physically, mentally, financially….all of it was impacted. No one should have to go through that when a newborn screening tool exists. It would have prevented so much.

In May of 2024, she wrote that she was happy vs. neutral for the first time in over a year. The medication is working.

The retail cost of a 90 day supply of Trientine is $22,700 per child. Thank God we have good insurance. I shudder to think of those that have to choose between paying their electric bill and getting life saving medication.

It has been 7 months since she started chelation. She still has a ways to go, improvements are there. Small things are huge. The hypomimia has improved, I can hear her sing in the car to the radio. I tear up every time. As I write this, we are on our first vacation in 2 years. She is able to participate in so much more these days. She will hopefully get to participate in her last year of Junior High with support.

Carrier status is 1:90. I suspect the incidence of Wilson's Disease is much higher than it is diagnosed. The reality is that it is treatable in most instances. A newborn screening tool would have prevented so much for all of us.

My daughters have exhibited a strength that some adults may struggle with. I cannot express how proud I am of them. We will be advocates for this disease and try to help those navigating it as we navigate it ourselves. Brinley often tells me that she wants her story told.

I thank you for your time and consideration.

Sincerely,

Nora Closser
noratoole@hotmail.com
585-737-5323

Erin Brooks
4907 N Wolcott Avenue, Unit 2B
Chicago, IL 60640

July 25, 2024

Board of Directors
Washington Department of Health
Town Center 2
111 Israel Rd. SE
Tumwater, WA 98501

To Whom It May Concern:

My name is Erin Brooks, and I am writing to urge you to add to add Wilson Disease (WD) to the Washington State newborn screening panel.

My father passed away from Wilson Disease in 2000. At the time, Wilson Disease was a highly unknown disease with minimal resources and options for treatment. It took over a year for my dad to be diagnosed and although there were wonderful doctors overseeing his care, the disease was too much for my dad's body to handle.

Over the past 24 years there have been so many advances made in the diagnosis and treatment for Wilson Disease and if caught early, WD patients and families can live normal and healthy lives. I am so grateful for Dr. Sihoun Hahn's efforts at Seattle Children's Hospital to develop this screening test for WD and lead the pilot project to test this assay on 25,000 newborns in Washington. If this test had been available for my father, his Wilson's would have been discovered long before it took such a serious toll on his body, and he may still be with us today.

So once again, please seriously consider adding Wilson Disease to your newborn screening panel. This is a life-or-death screening for many families.

With deep gratitude,
Erin K Brooks

Dear Dr. Han,

I wasn't diagnosed with Wilson's season 226 mate feeling my second year of medical school at Texas Tech health science Center. I had a Trevor and a grin. Trevor was getting worse and I thought God was punishing me for various events that I thought I was guilty of

It was missed by three expert Neurologist over prior 15 years! It is rarely seen by any neurologist or hepatologist and both kinds of doctors just overlook it. It's inevitable that they will overlook it because I just made you see one case and they're alive If That many?

Hence, with modern medical testing of a newborn DNA would easily diagnose the Wilson's disease gene along with all the other genetic disorders that are diagnosed Postpartum.
Like hemochromatosis ,hemophilia, et al.

Ok ? I feel just Adding Another Fault in a babies DNA the Cost would be in saving and making a life hell of a lot better as well as saving the cost of a liver transplant, which is in the hundreds of thousands of dollars!

In my case it was the cost of the college education and medical school education expenses, while living. Giving up of my dreams of practice medicine and field psychiatry where we know psychiatrist are all over the country. I took disability insurance while I had it. Dammit, That was when I was 40.

Thank You,

Dr Kirk Vestal MD

Date: July 25, 2024

To: WA State Department of Health, Board of Directors

re: My personal story with Wilson Disease

I first started experiencing symptoms of Wilson Disease when I was 20 years old while attending the University of Washington. My symptoms included tremors in my hands and arms, difficulty walking, and slowed speech. My symptoms became so bad that I had to drop out of the University and move back in with my parents.

My parents and I went to see a doctor so that we could find out what was causing my symptoms. After undergoing several medical tests, the doctor misdiagnosed me as having multiple sclerosis. For approximately five years, I lived with this misdiagnosis, while my symptoms kept getting worse, especially the tremors. During this five year period, in order to help correct the tremors, I underwent a type of brain surgery, called a thalamotomy. The surgery was a success, as it definitely helped lessen the tremors in my arms and hands. After the surgery, I did have to go through lengthy physical and occupational therapy to regain my body's full motor skills, in what I referred to at the time as "learning how to walk again".

After the five years of my misdiagnosis of multiple sclerosis, and while undergoing a routine eye exam, I found that I had a brown colored ring around my cornea. The eye doctor informed me that this was unusual and suggested I get it checked out by my doctor. With this new information, I went to see my neurologist. After undergoing some medical tests, I was correctly diagnosed as having Wilson Disease. I then was prescribed the correct medication, cuprimine, to help manage the excess amount of copper in my body. Within about six months of treatment most of my original symptoms of Wilson Disease greatly improved.

I was not correctly diagnosed with Wilson Disease until I was 25 years old (I am currently 61), so my life could have benefited from an earlier detection of the disease. My story shows how important it is to have Wilson Disease as part of mandatory newborn screenings.

Sincerely,

Thomas Sandall

Marilee & Gary Wolter
7324 Lantana Way
Naples, FL 34119

To: Washington Department of Health, Board of Directors,

My name is Marilee Wolter, my husband and I are writing to you to join in the advocating of newborn screening for Wilson disease (WD).

Our daughter Rhonda Rowland was diagnosed at the age of 21 with Wilson disease in Madison, WI by a new gastroenterologist. Looking back, we know she was incredibly lucky that this young physician thought of WD.

Six months prior to her diagnosis, she developed sudden, severe abdominal pain that kept her in bed so she couldn't attend her college classes. We brought her to our family internist in Milwaukee who attributed her abdominal pain to drinking bad water during a recent trip to Florida. Because of abdominal pain and fatigue, she ended her college junior year on academic probation. That summer she had extensive fatigue, experienced moodiness and made careless mistakes at her summer job. By September she developed jaundice, swelling, ascites and cirrhosis of the liver. Fortunately after receiving the correct diagnosis, she responded well to WD medication and has lived a normal life.

In 2016, my husband and I joined Rhonda at a Wilson Disease Association conference. We were shocked and saddened to see the devastating effects of WD. There were people in wheelchairs or walked with difficulty; many people couldn't speak clearly or control their verbal outbursts. Some needed assistance with eating. I couldn't believe they had the same disease as our daughter and we realized just how lucky we were.

Fortunately her younger sister and brother do not have Wilsons. Rhonda is in good health, and has dedicated the last 41 years of her life reporting medical information and raising awareness of this rare disease. We are proud of her work as vice president of the Wilsons Disease Association.

We strongly encourage the Washington Dept. of Health, Board of Directors, to consider and include WD in its newborn screening panel. While life-threatening if not detected, it's no longer a "rare" disease to those who experience it!

Thank-you for your support.

Best Regards,
Marilee & Gary Wolter

Sirs:

I read today that you have developed a screening test for WD. You plan to present letters from patients, caregivers and supporters reinforcing the importance of adding WD to Washington's newborn screening panel.

We live in Highland Park, IL. Our daughter, Hilary, was diagnosed with WD at age 25.

Before she was diagnosed, since she was little, she suffered from stomach pains, illnesses that could not be explained, and general discomfort. WD was not suggested by any doctor. We are not surprised; it is so rare. Her doctors attributed it all to stomach and intestinal issues. Hilary managed to do everything (school, activities, summer camp, college, graduate school) but too frequently with great discomfort. After graduating with a master's degree from NYU, she stayed in New York.

She became extremely sick, exhausted and incapacitated while living in NYC. Doctors gave her disparate diagnoses; one even thought she could be cured with marijuana. Unable to work, she was terrified at the unknown nature of her problems. We dropped everything to join her to try to find out what was going on.

We were led to the wonderful Dr. Dietrich and his PA Helen Adams at Mt Sinai Liver Institute. They diagnosed WD and saved her life. We learned to our shock that we are carriers of recessive genes for a disease we had never heard of. Much damage had already been done to Hilary's liver as a result of copper she could not eliminate. Thankfully, she did not need a liver transplant, but it was discussed. This all could have been avoided had there been a screening test at birth.

Hilary has been successfully treated for 5 years now and we have nothing but optimism for her future.

How much easier her life would have been had she been diagnosed at birth. Please add WD to the newborn screening panel.

Maxine and Michael Bonn

350 North Deere Park Drive West

Highland Park, IL. 60035

mmbonn@gmail.com

To: Washington Department of Health, Board of Directors

I am writing in support of adding the screening test for Wilson's Disease to the newborn screening. Our son was diagnosed with Wilson's Disease on his 24th birthday. He is now 26 and is fully chelated and healthier than ever. That is the good news. The bad news is all of the years that he (and we) suffered with his symptoms, mostly psychological and behavioral. Again, those symptoms are greatly improved now that he is chelated.

When he was a teenager, he had elevated liver enzymes in his bloodwork results. Physicians assumed that it was caused by the medicines he was on because of his psychiatric issues, and they did not assess for other possible reasons. No one considered Wilson's Disease, and we had never heard of it. Just think how different his life (and ours) would have been if he had been diagnosed (and treated) beginning at birth. It would have saved years of heartache for all of us, and he would not have to deal with cirrhosis of the liver for the rest of his life.

Please begin testing all children at birth for this serious disease so that no one else has to experience the sometimes permanent damage caused by Wilson's disease. Thank you for your consideration.

Janet Laubgross, PhD

Clinical Psychologist

July 24, 2024

Board of Director
Washington State Department of Health

I'm writing in support of Key Proteo and Dr. Sihoun's Hahn's newborn screening test for Wilson disease. I urge the Washington Department of Health to add Wilson disease to your state's newborn screening panel.

Please indulge me as I share situation of people with Wilson disease in the place where I live.

My family's journey with Wilson's disease began in 2011. My older daughter, then 9 years old, often complained of abdominal pains. At the beginning we thought this was related to the stress of starting primary school in 2009 and the arrival of her younger sister at the same time. The recurring complaints prompted our physician to extend the diagnostics, which showed high levels of liver tests. For the next six months, doctors observed and monitored my child. Unfortunately, there was liver damage during this period, so we decided to transfer our daughter to another hospital in December 2010. Fortunately, experts in Wilson's disease were working there and in January 2010 we had a diagnosis and treatment began. In February 2011, my younger daughter, who was then 2.5 years old, was also diagnosed.

I run a support group on Facebook for people with Wilson's disease, which currently has 270 patients or relatives as members. A year ago, a dad of a 16-year-old girl who had been diagnosed with Wilson's disease four days earlier wrote in the Facebook support group. The girl required an urgent liver transplant. She had brain swelling and was put into a pharmacological coma. Her liver was transplanted but unfortunately this child's life could not be saved. There is also another student. She is her mother's second child, the first died undiagnosed. There are several more similar stories...

I would like to emphasize that in Wilson's disease, the key issue is the earliest possible diagnosis. Unfortunately, knowledge of the disease among people and physicians is still very low. The introduction of screening could save many lives and certainly dramatically improve diagnosis and treatment and thus prevent side effects and improve the quality of life of those suffering from this disease.

I am in the process of registering a patients' association, of which I will be president. I am in contact with patients from many countries and I intend to take any action that improves the quality of life of people affected by this disease.

As patients and their families, we look forward to organized early diagnosis, to screening. We look to the future with hope. We have high hopes that Washington State will show leadership and courage and stand the first jurisdiction to add NBs for Wilson disease to its newborn screening panel and become in the future an example for other states and countries.

Best regards,
Anna Aniol
Wroclaw, Poland



Board of Directors

Washington State Department of Health

P.O. Box 47890

Olympia, WA 98504-7890

29th July 2024

Dear Board Members,

I am writing to express my strong support for Dr. Sihoun Hahn's newborn screening test for Wilson Disease (WD) and to urge you to consider adding this critical test to your screening panel. Including Wilson Disease in the newborn screening programme could spare many families the devastating consequences of a late WD diagnosis, as my family has endured.

Please allow me to share my daughter Sophie's story, which underscores the urgent need for early detection. Sophie was a happy, (seemingly) healthy child and teenager who excelled in her studies, and she was a talented pianist.

At the age of 15, Sophie experienced a sudden and dramatic personality change accompanied by severe psychiatric symptoms. Like many WD patients, her diagnosis was delayed by about a year from the onset of symptoms and she deteriorated significantly during this time. Sophie is now 19 years old and has spent the last four years in mental health institutions or acute healthcare settings. She hasn't spent a Christmas day at home since she was 14. Sophie's condition has worsened over time. In addition to her initial psychiatric symptoms, she now suffers from hepatic and neurological problems.

Sadly, she has also developed cognitive deficits due to the WD brain damage she has suffered, and it is nothing short of a tragedy that she was unable to complete her education, receiving no qualifications despite being a straight A student. She had always dreamed of becoming a paediatric nurse. Albeit minor in the scheme of things but of significant magnitude to Sophie, sadly, she can no longer play the piano.

Sophie now requires 24-hour care and is under a Deprivation of Liberty order. The once incredibly witty, smart, vibrant, and talented girl I knew has been stripped of everything that brought her joy or function in life. She now has an extremely poor quality of life, and despite four years of treatment with penicillamine, there has been no improvement in her condition. I fear she may never recover, but I will never lose hope.

In Sophie's words: "Mum, it just isn't living."

During a recent visit to our local emergency department, a physician greeted her with, "*I don't know how you are still alive!"* This stark comment underscores the severity of her condition. This is our reality now - a reality I am forced to accept, but if she had been diagnosed as a baby, *oh, how different things would be*!

I would give anything to have known she had WD as a baby. This profound loss and suffering could have been prevented. There are other children out there right now who have WD but don't know it yet, and devastating consequences are looming. This thought keeps me awake at night.

You have the power to prevent other children in your state from suffering the profound, relentless, enduring suffering and loss that we do, every single day. You have the power to save lives.

Thank you for kindly reading about Sophie and for your consideration of this critical matter.

Yours sincerely,

Claire Stapleton

Mother of Sophie, WD Patient

England, UK

E: claire.stapleton1@outlook.com

T: +44 7917 823241



WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Panel PROCESS & CRITERIA

PROCESS TO EVALUATE CONDITIONS FOR INCLUSION IN THE REQUIRED NEWBORN SCREENING PANEL

Last updated March 14, 2025



WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Panel PROCESS & CRITERIA

The Washington State Board of Health (Board) has the duty under RCW 70.83.050 to define and adopt rules for screening Washington-born infants for heritable conditions. Chapter 246-650-020 WAC lists conditions for which all newborns must be screened. Members of the public, staff at Department of Health (Department), and/or Board members can request that the Board review a particular condition for possible inclusion in the newborn screening (NBS) panel.

To determine which conditions to include in the NBS panel the Board convenes a newborn screening technical advisory committee (TAC) to evaluate candidate conditions using guiding principles and an established set of criteria.

This document describes the Qualifying Assumption, Guiding Principles, and Criteria the Board has approved to evaluate conditions for possible inclusion in the newborn screening panel. The Board and Department apply the qualifying assumption. The Board-appointed Newborn Screening TAC applies the following three guiding principles and evaluates the criteria to make recommendations to the Board on which conditions to include in the state's required NBS panel.

QUALIFYING ASSUMPTION

Before the Board convenes a TAC to review a candidate condition against the newborn screening criteria, staff complete a preliminary review to determine whether sufficient scientific evidence is available to apply the criteria for inclusion, which is the qualifying assumption. If the candidate condition is on the Health Resources and Services Administration (HRSA) Recommended Uniform Screening Panel (RUSP), the Board and Department will consider the qualifying assumption met and convene a TAC.

A note on the RUSP: The RUSP is a list of conditions that the Secretary of the Department of Health and Human Services (HHS) recommends that states screen for as part of their newborn screening programs. Once the HHS Secretary recommends a new condition, the Board and Department will review it for possible inclusion in the Washington NBS panel within two years of the recommendation.

Conditions pending RUSP Review or Previously Denied for the RUSP: RCW 34.05.330 of the Administrative Procedures Act (APA) allows any person to petition a state agency to adopt, repeal, or amend any rule within its authority. Agencies must respond to the petitioner within 60 days. If the agency accepts the petition, it must initiate rulemaking. An agency can deny the request for rulemaking, and in doing so, it must explain its reasons and, if appropriate, describe alternative steps it is prepared to take.

If the Board receives a petition for rulemaking regarding a candidate condition currently under review for the RUSP, the Board will wait until the federal committee finishes its review and the HHS Secretary makes a final decision before convening a TAC. For petitions involving conditions that have already been reviewed and denied inclusion on the RUSP, the Board will instruct staff to work with the petitioner to determine if concerns raised during the federal review have been addressed before recommending the Board convene a TAC to review the condition.



WASHINGTON STATE BOARD OF HEALTH

Newborn Screening Panel PROCESS & CRITERIA

THREE GUIDING PRINCIPLES

Three guiding principles govern all aspects of the evaluation of a candidate condition for possible inclusion in the NBS panel.

- A decision to add a screening test should be driven by evidence. For example, test reliability and available treatment have been scientifically evaluated, and those treatments can improve health outcomes for affected children.
- All children who screen positive should have reasonable access to diagnostic and treatment services.
- Benefits of screening for the disease or condition should outweigh harm to families, children, and society.

CRITERIA

1. **Available Screening Technology:** Sensitive, specific, and timely tests are available that can be adapted to mass screening.
    - The sensitivity of the screening test is estimated to be ≥95%.
    - The specificity of the screening test is considered acceptable based on the estimated number of false positive results and their potential impact on the families, healthcare system, and newborn screening program.
    - A timely test is one that enables intervention before irreversible harm develops, within the current standard timeframes for specimen collection, receipt, testing, and reporting.
    - There is adequate peer reviewed evidence to evaluate this criterion.
2. **Diagnostic Testing and Treatment Available:** Accurate diagnostic tests, medical expertise, and effective treatment are available for evaluation and care of all infants identified with the condition.
    - A diagnostic test accurately identifies who needs treatment and is readily available to all newborns screened.
    - The available treatment is effective in reducing morbidity or mortality and outweighs any risks or harms of the treatment.
    - The medical expertise needed to diagnose and care for those with a positive newborn screen is reasonably available to all newborns screened.
    - The appropriate consultants and treatment centers have been identified and have capacity for the expected increase in diagnostic testing or referrals.
3. **Prevention Potential and Medical Rationale:** The newborn identification of the condition allows early diagnosis and intervention.
    - There is sufficient time between birth and onset of irreversible harm to allow for diagnosis and intervention.
    - The condition must have an onset form that occurs in infancy (within the first year of life); newborn screening is not appropriate for conditions that only present after the first year of life.
    - The benefits of detecting and treating infantile-onset forms of the condition (within one year of life) balance the impact of detecting later onset forms of the condition.
    - There is adequate evidence of acceptable quality to evaluate this criterion.



4. **Public Health Rationale:** The nature of the condition justifies population-based screening rather than risk-based screening or other approaches.

- All available risk-based screening tools for the condition have been considered and are found to be inferior to universal newborn screening.
- There is adequate evidence of acceptable quality to evaluate this criterion.

5. **Benefit-cost/Cost-effectiveness:** The outcomes outweigh the costs of screening. All outcomes, both positive and negative, need to be considered in the analysis.

- The economic analysis considers:
  - The prevalence of the condition among newborns
  - The positive and negative predictive values of the screening and diagnostic tests
  - Variability of clinical presentation by those who have the condition
  - Dollar values for costs and benefits of screening vs. no screening
- The impact of ambiguous results, adverse effects, or unintended consequences of screening, such as psycho-social or economic impacts on the family and medical system, must also be considered.
- The results of the economic analysis shows that the outcomes, financial or otherwise, outweigh the costs of screening.
- There is adequate evidence of acceptable quality to evaluate this criterion.

6. **Public Health Infrastructure Readiness:** The Newborn Screening Program's capacity to implement screening within a reasonable time frame must be considered.

- The systems and staffing necessary to perform the test and report screening results have been identified.
- Resources needed to implement short- or long-term follow up protocols by the newborn screening program have been identified.
- Accessibility to treatment for anyone diagnosed with the condition is considered acceptable based on the frequency of treatment needed.

RCW 70.83.020

Screening tests of newborn infants.

(1) It shall be the duty of the department of health to require screening tests of all newborn infants born in any setting. Each hospital or health care provider attending a birth outside of a hospital shall collect and submit a sample blood specimen for all newborns no more than forty-eight hours following birth. The department of health shall conduct screening tests of samples for the detection of phenylketonuria and other heritable or metabolic disorders leading to intellectual disabilities or physical defects as defined by the state board of health: PROVIDED, That no such tests shall be given to any newborn infant whose parents or guardian object thereto on the grounds that such tests conflict with their religious tenets and practices.

(2) The sample required in subsection (1) of this section must be received by the department [of health] within seventy-two hours of the collection of the sample, excluding any day that the Washington state public health laboratory is closed.

[2014 c 18 § 1; 2010 c 94 § 18; 1991 c 3 § 348; 1975-'76 2nd ex.s. c 27 § 1; 1967 c 82 § 2.]

RCW 70.83.030

Report of positive test to department of health.

Laboratories, attending physicians, hospital administrators, or other persons performing or requesting the performance of tests for phenylketonuria shall report to the department of health all positive tests. The state board of health by rule shall, when it deems appropriate, require that positive tests for other heritable and metabolic disorders covered by this chapter be reported to the state department of health by such persons or agencies requesting or performing such tests.

[1991 c 3 § 349; 1979 c 141 § 113; 1967 c 82 § 3.]

RCW 70.83.050

Rules and regulations to be adopted by state board of health.

The state board of health shall adopt rules and regulations necessary to carry out the intent of this chapter.

[1967 c 82 § 5.]



Health Impact Reviews

Miranda Calmjoy (she/they)
Lindsay Herendeen (she/her)
Cait Lang-Perez (she/her)

August 20, 2025

WASHINGTON STATE BOARD OF HEALTH

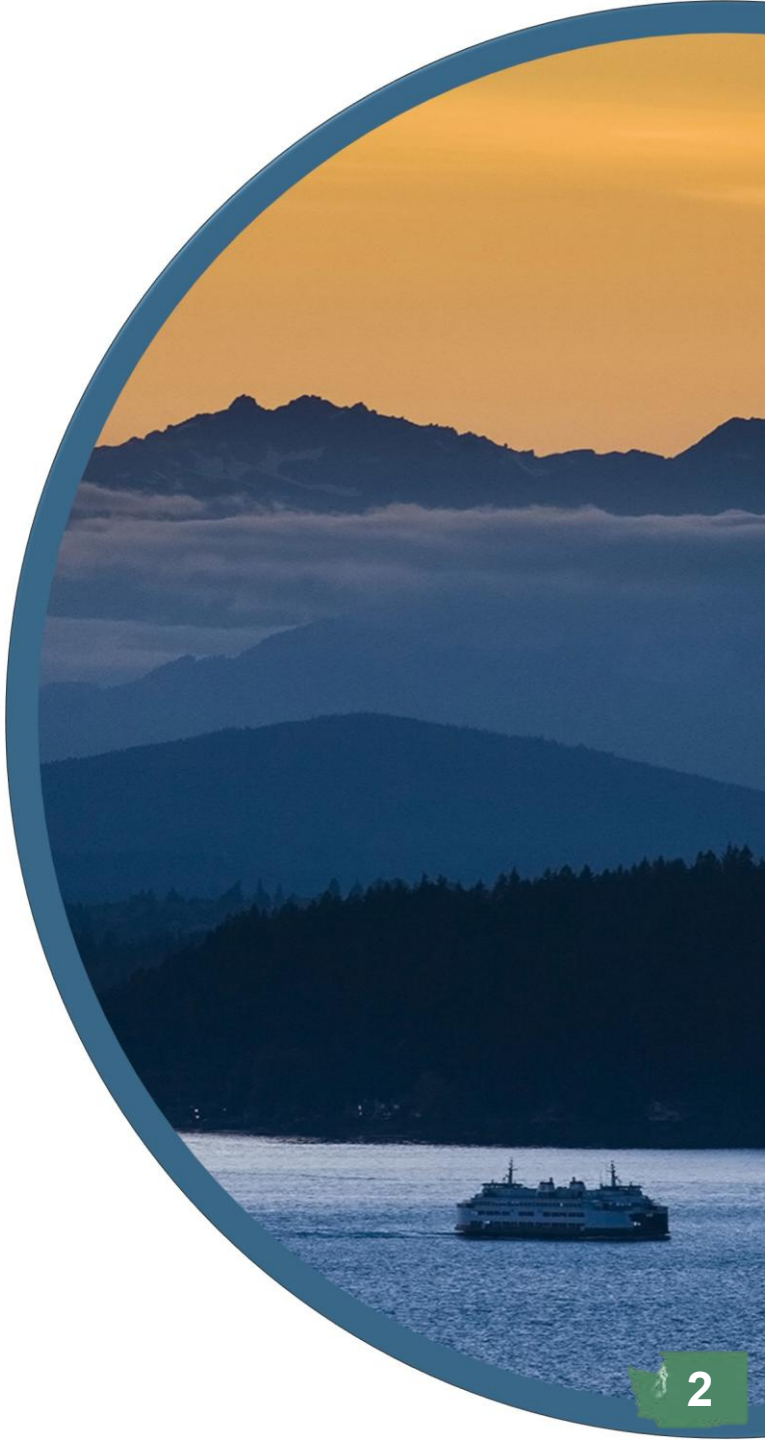
HEALTH EQUITY
Governor's Interagency Council on Health Disparities

Health Impact Reviews (HIR)

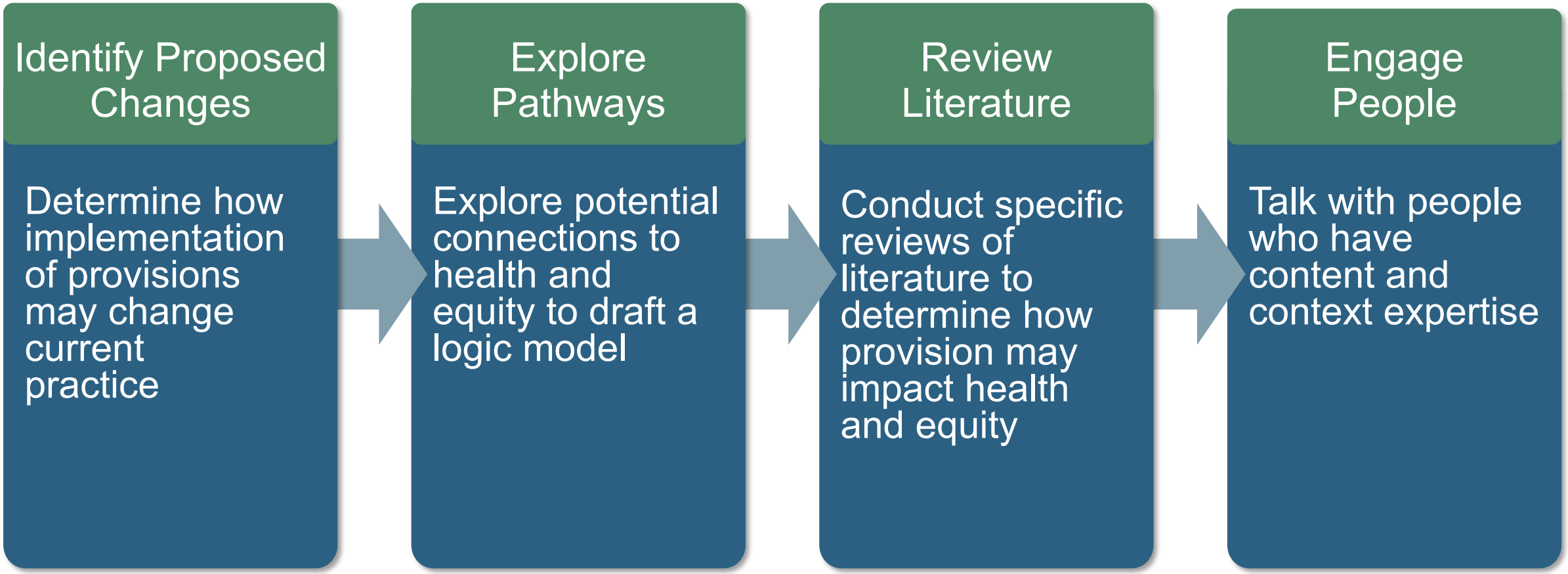
HIRs can be requested for any topic.

- **Objective, nonpartisan, evidence-based analysis**
- Prospective tool
- Determine how a legislative or budgetary change will likely impact **health** and **equity**
- Requested by any state lawmaker or the Governor
- Must be completed in 10 days during legislative session

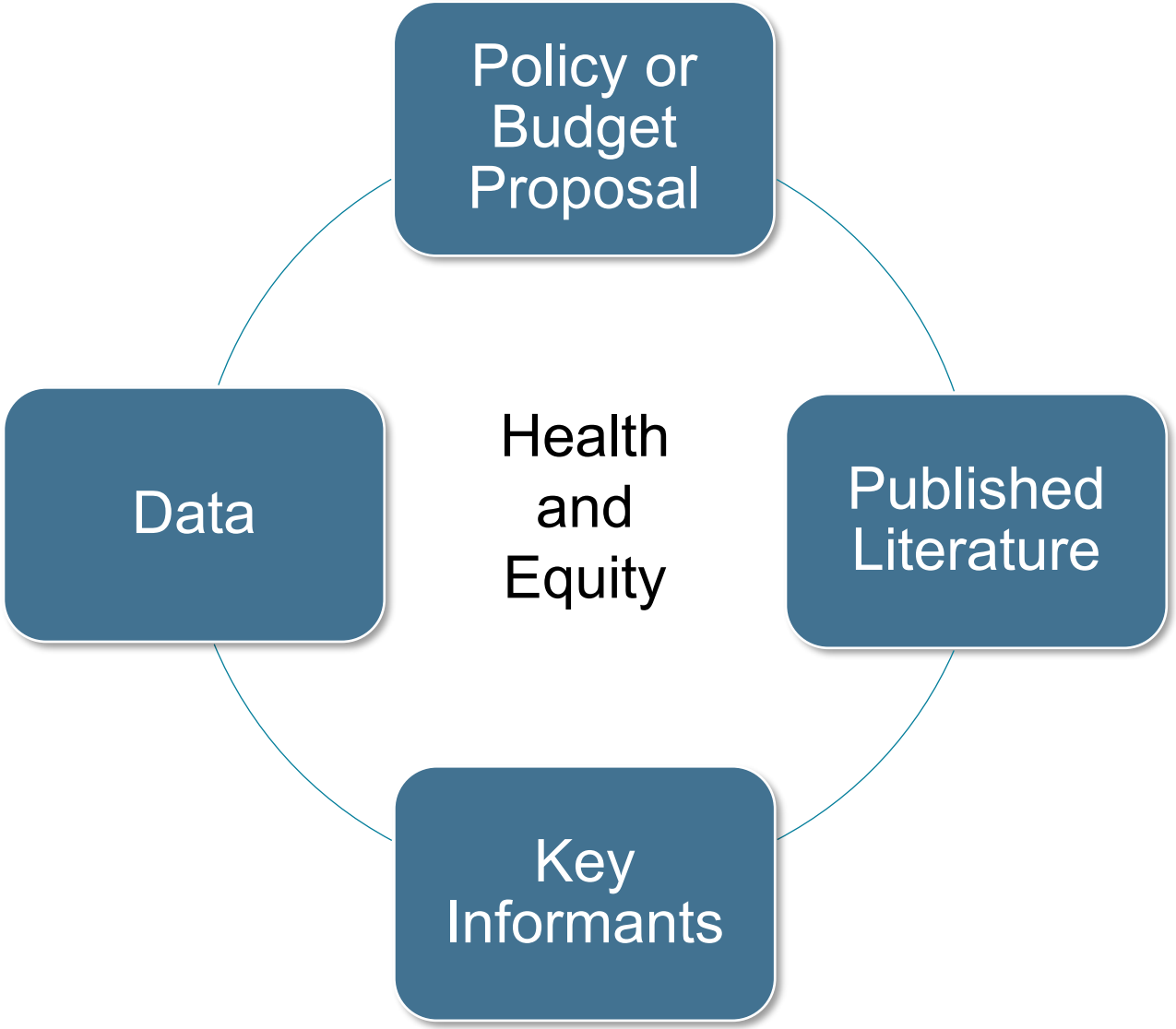
(RCW 43.20.285)



HIR Process


Identify Proposed Changes
Determine how implementation of provisions may change current practice
Explore Pathways
Explore potential connections to health and equity to draft a logic model
Review Literature
Conduct specific reviews of literature to determine how provision may impact health and equity
Engage People
Talk with people who have content and context expertise

Contributing Information



Types of Key Informant Engagement



Strength-of-Evidence Criteria

Ratings are based on criteria which consider:

- The amount of research
- Appropriateness of study design
- Study execution
- Generalizability



VERY STRONG EVIDENCE

STRONG EVIDENCE

A FAIR AMOUNT OF EVIDENCE

EXPERT OPINION

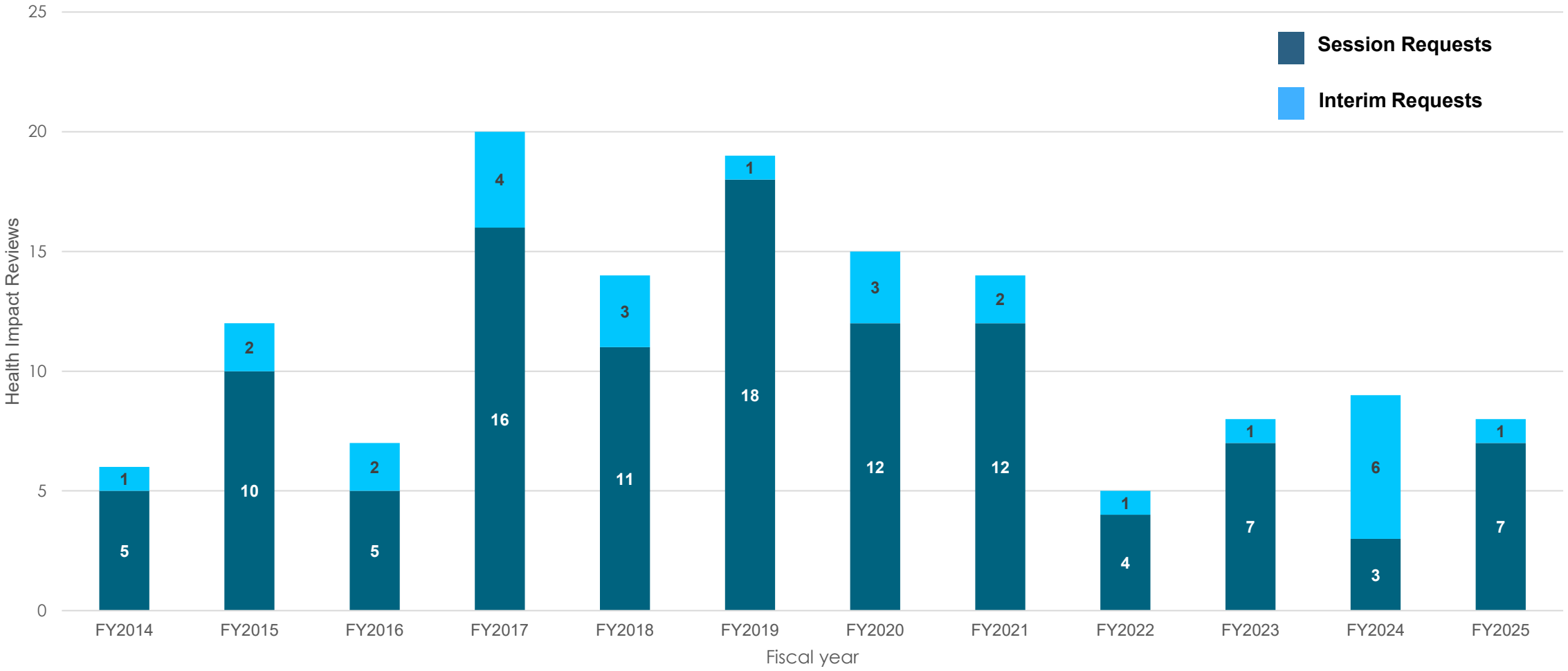
INFORMED ASSUMPTION

NO ASSOCIATION

NOT WELL RESEARCHED

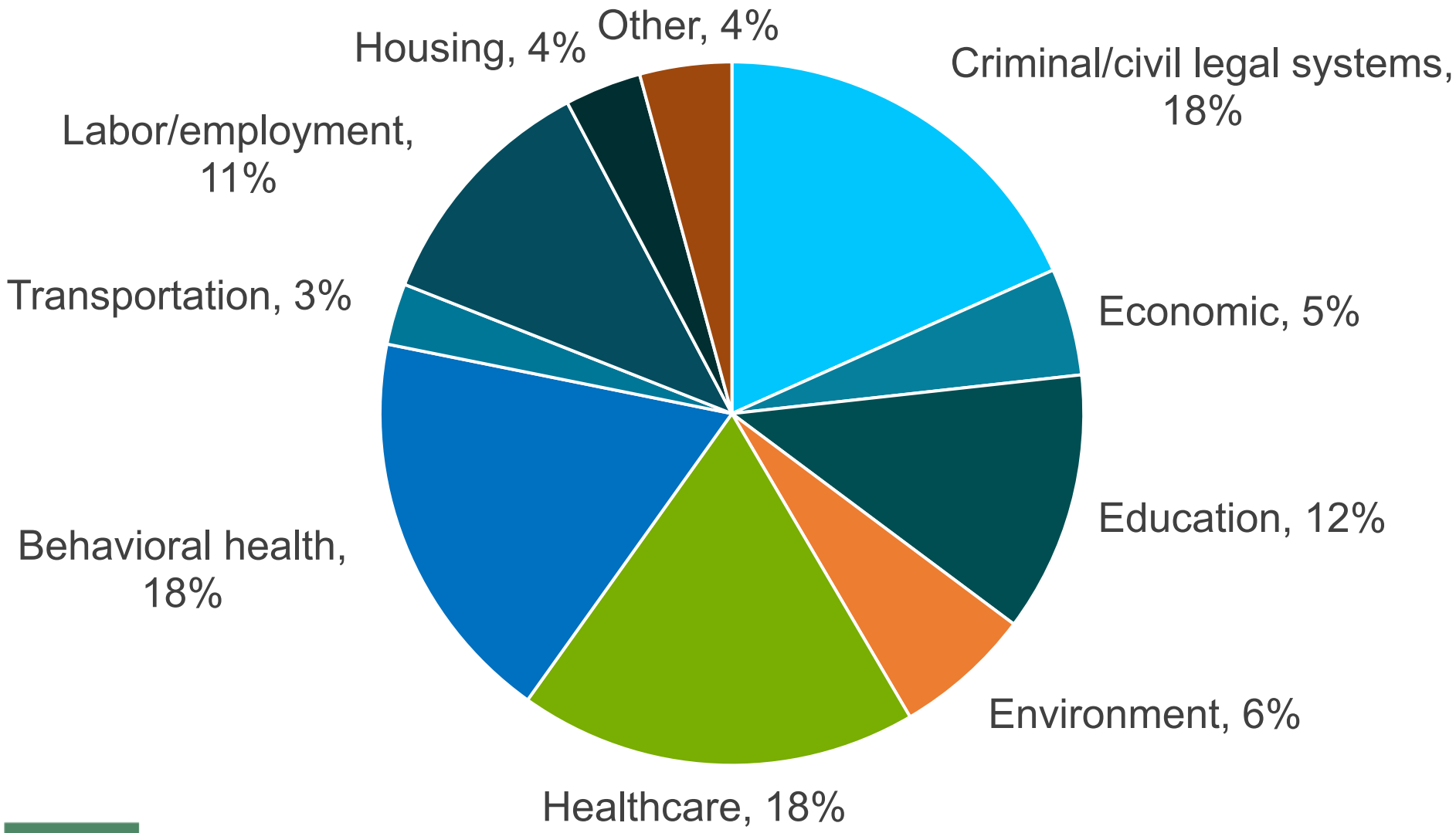
UNCLEAR

137 Completed HIRs



HIR Requests by Topic Area

Bill topics analyzed 2014-2025 (n=137)



Fiscal Year 2025 (FY25) by the Numbers

8 The **number of Health Impact Reviews** completed during FY25.	**124** The **number of key informants** engaged as part of HIRs completed in FY25.
88% The **percentage of FY25 HIR requests** made during the 2025 Legislative Session.	**85%** The **percentage of key informants** (92 of 108) engaged during Legislative Session who were new contacts.
54% The **percentage of legislators** who staff engaged as part of HIR outreach efforts during FY25.	**1** The **number of HIR-related bills that passed** the Legislature in 2025 and were signed into law by the Governor.

SHB 1606 (Chapter 64, Laws of 2025)

- SHB 1606, Concerning state employee access to peer-reviewed journals
- Sponsored by Representative Engell
- Directs Washington State Institute for Public Policy (WSIPP) to conduct a study by June 30, 2026, of potential funding, organizational structure, and policy mechanisms to provide state employees with electronic access to peer-reviewed journals.
- Supported by the Washington State Board of Health and Governor's Interagency Council on Health Disparities.



How HIRs Inform Policy

Requesters have used HIRs to understand:

- The evidence base for a proposal
- If a proposal will have the intended impact
- Potential unintended consequences
- Equity implications

Requesters have used HIR findings to:

- Talk with colleagues about a proposal
- Refine a policy
- Discuss the bill on the floor
- Develop points for budget negotiations
- Inform their vote on a bill



2025 Feedback from Legislators

Legislators used and valued HIRs to:

- **Test assumptions** of bills as drafted
- Help lay out a **workplan** to refine a proposal
- Summarize available research which can help show the **impacts of investment**
- Have data and information in one place as a **central reference document**
- **Build trust** with community and advocates
- Be non-partisan, evidence-based, and a **reliable and trusted source** of information
- Be a Washington State product and include **state-specific** information

2025 Key Informant Interviewee Feedback

Role	Total
WA State Agency	17
Community-Based Organization	4
Professional Association	3
Individual, Community Member	2
Agency outside of WA	0
Researcher	0
Total	26 (21%)

Of key informants who responded…

- **96%** said participation was worthwhile
- **23%** reported using HIR findings

Ways HIR findings were used:

- Education
- Advocacy

Most useful about HIR findings:

- The data included in the review
- Nonpartisan review
- Findings provided a different way to think about the policy
- Explored intersections with the overall policy landscape
- Illustrated the need for more research

National Connections

Public Health Law Center (PHLC) at Michell Hamline School of Law

- **Report:** Applying Racial & Health Equity Impact Assessments for Better Policy Making (March 2025)
- **Webinar:** Exploring the Impact and Challenges of R/HEIA Implementation: Stories and Insights from State and Municipal Staff (May 2025)

Center for Public Health Law Research at Temple University

Community of Practice: Legal Epidemiology (May 2025)

Network for Public Health Law (NPHL)

- **Panel Presentation:** Embedding Equity in State-Wide Decision-Making to Address Root Causes of Health Inequities (September 2025)



New Connections with State Researchers

State Agency	Work
Office of Financial Management, Public Safety Policy & Research Center (PSPRC)	• Serve as source for public safety data, research, policy, and statistical analyses. • Conduct Criminal Justice Racial and Ethnic Impact Statements (REIS) to identify potential disproportionate impacts of proposed sentencing-related policy changes on communities of color.
Office of the Washington State Auditor (SAO), Performance Audit Team	• Conduct performance audits to evaluate the efficiency and effectiveness of government programs. • SB 5414 (Chapter 135, Laws of 2025) requires SAO to consider social equity impacts during all performance audits.
Liquor and Cannabis Board (LCB), Research Program	• Conduct, analyze, and report internal and external research that is evidence-based and non-partisan related to the products, policy, and regulation of alcohol, cannabis, tobacco, and vapor products.



Contact the HIR Team

Miranda Calmjoy (she/they)
Lindsay Herendeen (she/her)
Cait Lang-Perez (she/her)

hir@sboh.wa.gov
360-628-7342

Completed Health Impact Reviews can be found on the Washington State Board of Health website:
https://sboh.wa.gov/health-impact-reviews

WASHINGTON STATE BOARD OF HEALTH

THANK YOU

To request this document in an alternate format, please contact the Washington State Board of Health at 360-236-4110, or by email at **wsboh@sboh.wa.gov** | TTY users can dial 711



Washington State Department of HEALTH

WATER RECREATION RULE REVISION

Water Recreation | Environmental Public Health

Presenter

Joe Graham

Manager

Water Recreation and Food Safety Section

Water Rec and Public Health

Illness outbreaks

- Nationwide, 40 outbreaks per year
- In Washington State, about one per year

Drowning

- Leading cause of death for children ages 1–4
- Second leading cause of unintentional injury death for children ages 5-14



Chapter 70.90 RCW – Water Recreation Facilities

Gives authority to adopt rules for:

- Design
- Operation
- Injury & Illness Reporting
- Biological & Chemical Contamination Standards
- Water Quality Monitoring
- Inspections
- Permit Application
- Enforcement

Water Recreation Facilities (WRF)

- About 4,800 WRF in WA
  - Swimming pools
  - Spas (hot tubs)
  - Splash pads
  - Water parks



Responsibilities

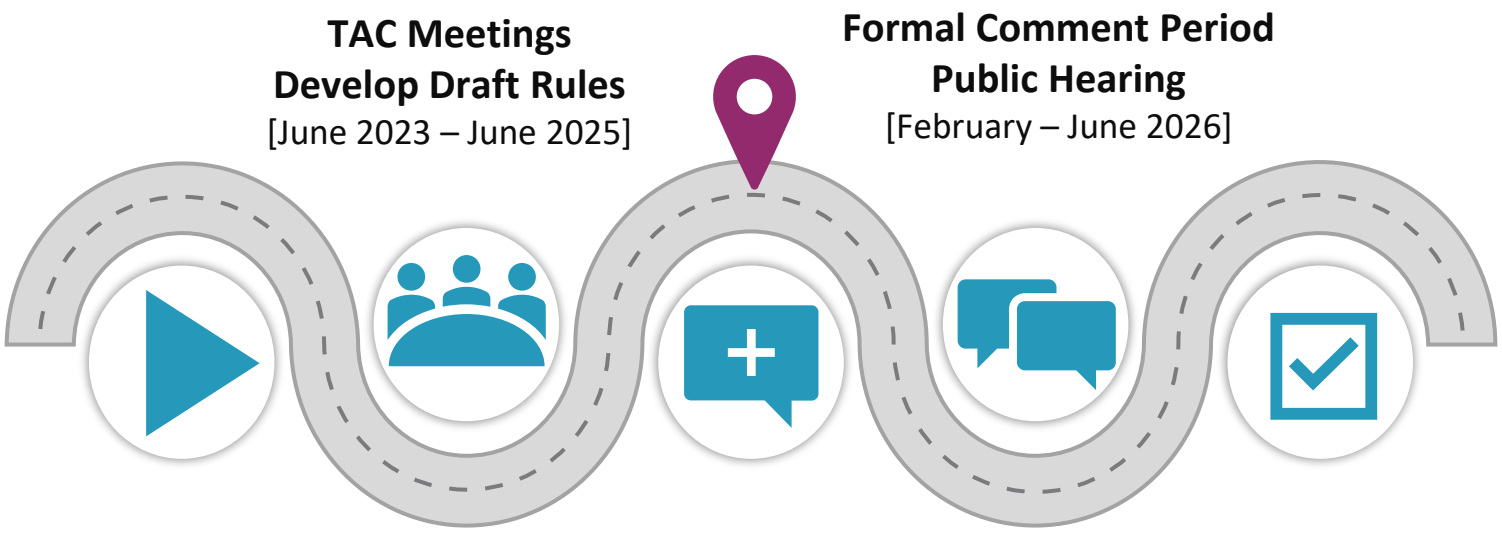
DOH

- Delegates authority
- Construction review
- Assist SBOH with rulemaking
- Support
  - Technical
  - Investigations
- Permitting and inspecting WRF in Lewis County

Local Health Jurisdiction

- Permitting and inspecting
- Construction review
- Investigating incidents
  - Illnesses
  - Injuries
  - Drowning

Rulemaking Timeline

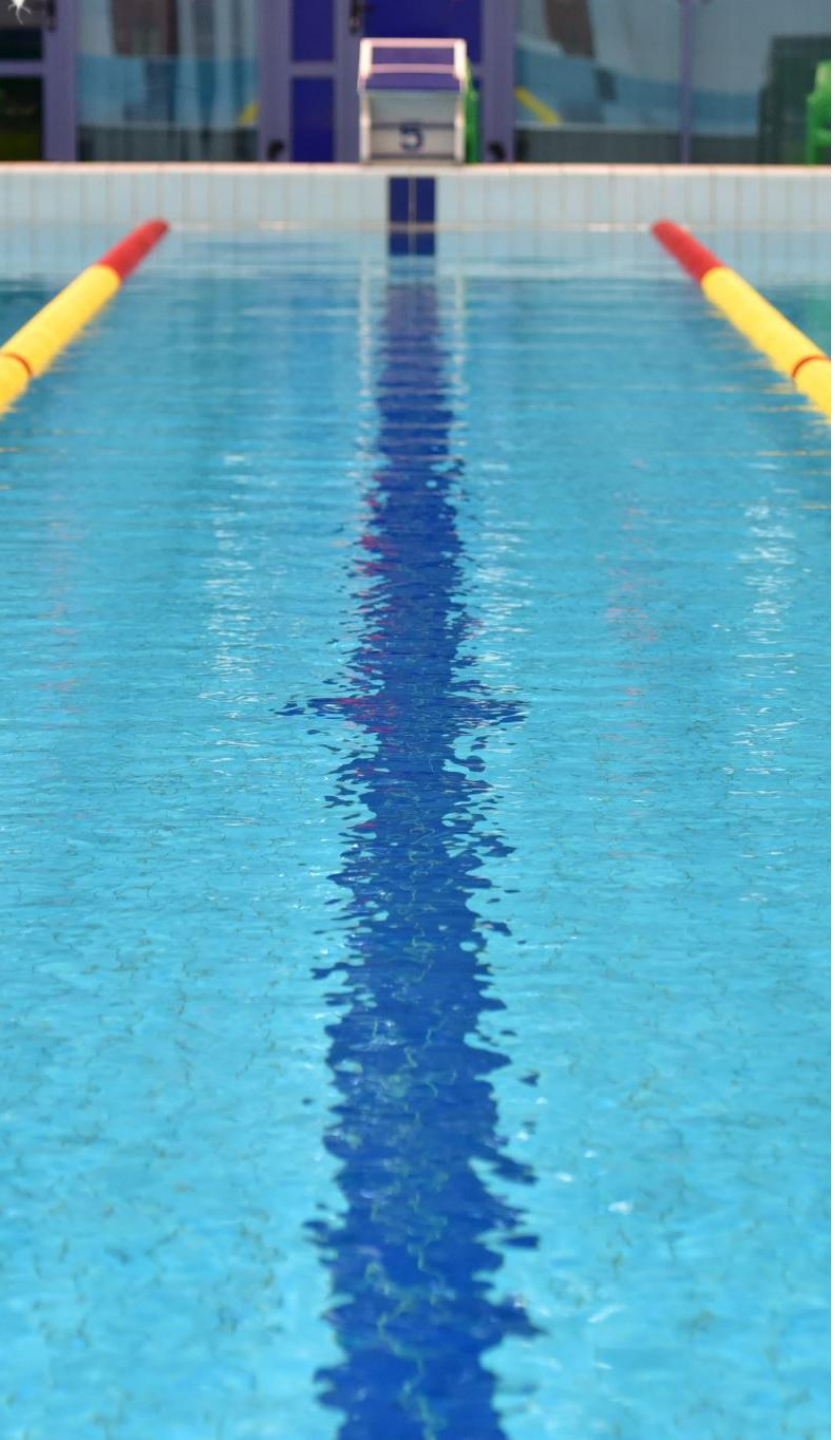


*Rulemaking paused from 2017-2023 due to staffing changes and COVID-19 response



Rulemaking Scope

- Consider current best practices
- Improve public health and safety
- Consolidate chapters for clarity



Rule Development

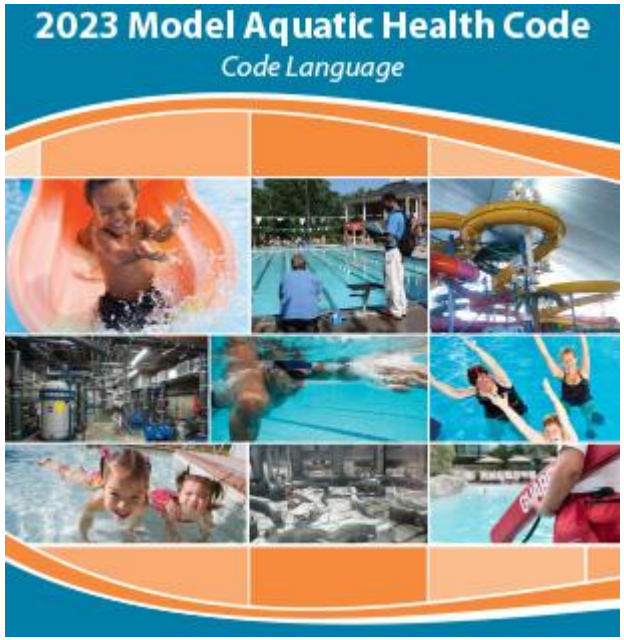
- Technical Advisory Committee
- Consideration of CDC Model Aquatic Health Code (MAHC)
- Rulemaking Petitions

Technical Advisory Committee

- Local health jurisdictions
- Pool and water park owners and operators
- Washington Hospitality Association
- Department of Labor & Industries
- Lifeguard certifying associations
- Washington Parks and Recreation Association
- Pool engineers and architects
- Pool and Hot Tub Association

CDC Model Aquatic Health Code (MAHC)

- Guidance to promote healthier and safer swimming and water activities
- Reviewed and evaluated the 2018, 2023, and 2024 versions
- Incorporated provisions based on TAC input and state law



Rule Petitions

- ADA compliance
- Lifeguard allowance



Key Themes & Discussion Topics

Most discussed topics:

- HVAC
- Color and finish of pools
- Pool side alcohol consumption
- Lifeguarding
- Imminent health hazards

Next Steps

- Informal public comment period closes on September 30, 2025
- Assess the comments and revise draft rule language
- Further engagement with interested parties as necessary
- Develop and publish a cost survey
- Begin drafting the Significant Analysis and Small Business Economic Impact Statement

Questions



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Washington State Board of Health

FY26-27 Budget Overview

Ashley Bell, Board Staff

August 20, 2025

WASHINGTON STATE BOARD OF HEALTH

Overview

- Historical funding
- Review of the fiscal year 25 (FY25)
- State budget outlook
- Zero-based budgeting
- Current biennium (FY26-27)
- Next steps
- Recap



Historical Funding by Biennium

- **2011**: One-time GFS reduction of $119,000 per fiscal year for health impact reviews
- **December 2011**: Early Action budget, SBOH was permanently reduced by $68,000 GFS per fiscal year

2011 – 2015

2015: GFS funding of $119,000 per fiscal year was restored for health impact reviews

2015 – 2017

2017: Received FPHS funding for .6 FTE (HIR)

2017 – 2019

- **2019**: Board received FPHS funding for maintenance .6 FTE (HIR) plus 1 FTE Policy Advisor)
- **2021**: Council received one-time project funding for the Office of Equity Task Force
- **2021**: Council received one-time project funding for the Environmental Justice Task Force

2019 – 2021

- **2021**: Received FPHS funding for maintenance funding of 1.6 FTE (Policy and HIR) plus 1 FTE Communications, and 1 FTE for an additional HIR analyst
- **2021**: Board received one-time project funding (.6 FTE Community Engagement Coordinator) for implementation of local board of health composition legislation

2021 – 2023

- **2022**: 2.6 FTE HIR reallocated from MA 4 to MA 5
- **2022**: .6 FTE MA3 reallocated to MA4 (community engagement coordinator)
- **December 2022**: Social Equity in Cannabis Task Force ended, final report submitted
- **2023**: 1 FTE CC2 reallocated to CC3
- **2023**: 1 FTE AA2 reallocated to an AA3

- **2023**: New funding for FTE Equity and Engagement Manager, Communication Consultant, Admin Coordinator, and to maintain .6 FTE Outreach Coordinator
- **2024**: Council budget increased, 4 staff hired

2023 – 2025

FY25 Appropriations

Funding	Total	Direct*
SBOH GFS	$1,281,836	$1,105,100
SBOH - Foundational Public Health Services (FPHS)	$1,179,698	$1,016,837
Health Disparities Council - GFS	$1,136,064	$976,000

One-Time Funding	Total	Direct*
SBOH - School Env Health & Safety Rules (Project)	$755,492	$654,203
SBOH - Newborn Screen BCKDK (Project)	$200,849	$174,000
SBOH – A/V (FPHS)	$50,390	$43,290

* After Department of Health agency indirect rates, which are 1.4% for contracts and 16.4% for non-contracts

Fiscal Recap of FY25

- One-time budget proviso ($750,004 total) for school health and environmental safety
- One-time budget proviso ($200,849 total) to review BCKDK and cCMV
- One-time FPHS investment ($50,000) for new Audio/Visual equipment for Board meetings
    - $43,290.00 direct from FPHS investment for new A/V equipment
    - $23,975.76 invested from GFS funding
    - $5,167.19 for language access that connects to the AV system-- Board FPHS engagement funds
    - $2,063.32 for an additional Spanish channel installed on all AV systems-- Board FPHS engagement funds
- Board member laptops $11,788.50 (GFS) to meet IT needs, and streamline public records requests

FY 2026 State Budget Outlook

- The State predicts a $750 million dollar shortfall
- Reduced federal funding increase demands on state services/ funding needs
- The 2024 funding increase for the Governor's Interagency Council on Health Disparities sustained a 50 percent reduction.
- Foundational Public Health Services funding was reduced by $22 million. Investment in the Board and Tribes remain unchanged.
- Department of Revenue projects a reduction to the foundational public health services account of $3.8 M in FY 2026, and $9.2 M in FY 2027.
- State agencies may be asked to identify additional reductions

Zero-Based Budgeting

- Requires every expense to be justified for each new period, starting with a “zero-base”
    - Resetting budgets to zero, effectively starting with a blank slate
    - Evaluating functions and teams and justifying and allocating expenses
    - Can be time-consuming and have a learning curve
- Advantages
    - Cost reduction and control
    - Improved resource allocation
    - Adaptability and flexibility
    - Enhanced operational efficiency
    - Increased transparency and accountability
- Will help navigate operating during a time where the state is expecting a large budget deficit
- Will help us understand our current and future needs

Annual Funding for FY26-28 Biennium**

Board GFS
Total Funding Allocated
$1,095,000.00

Council GFS
Total Funding Allocated
$509,000.00

Proviso GFS
Total Funding Allocated
$124,000.00

Engagement FPHS
Total Funding Allocated
$323,545.43

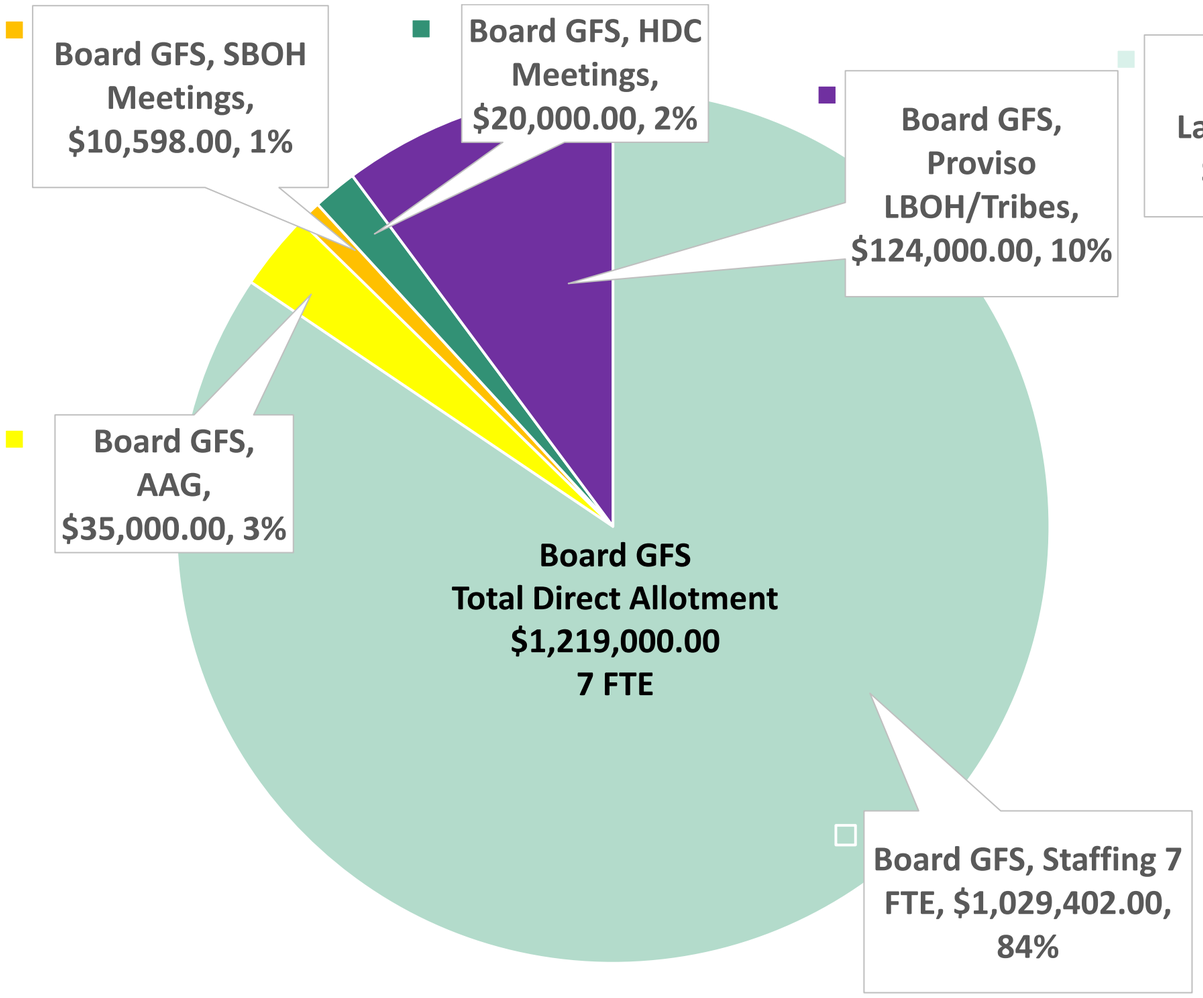
Comms and Policy FPHS
Total Funding Allocated
$448,264.57

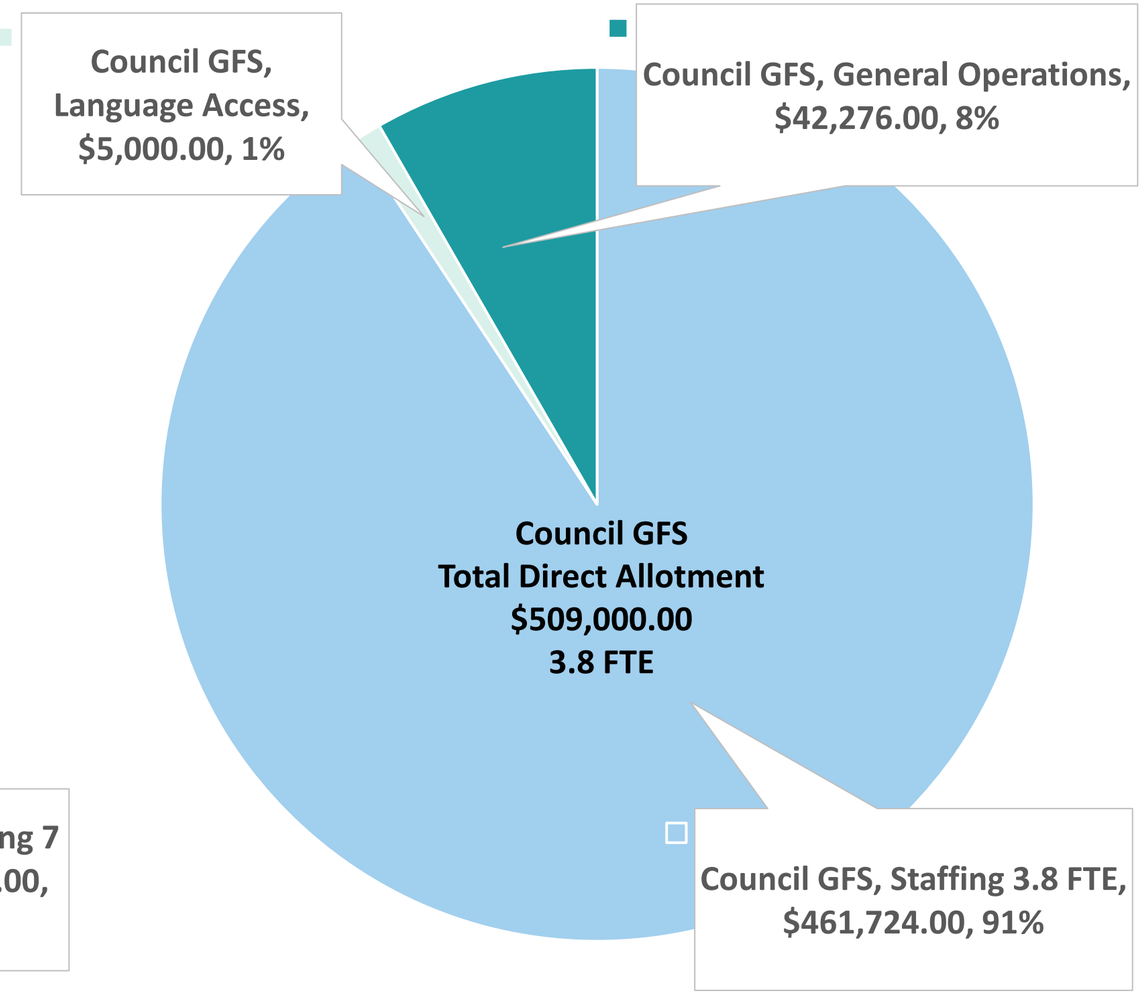
Health Impact Reviews FPHS
Total Funding Allocated
$245,027.00

All Funds
Total Funding Allocated
$2,744,837.00

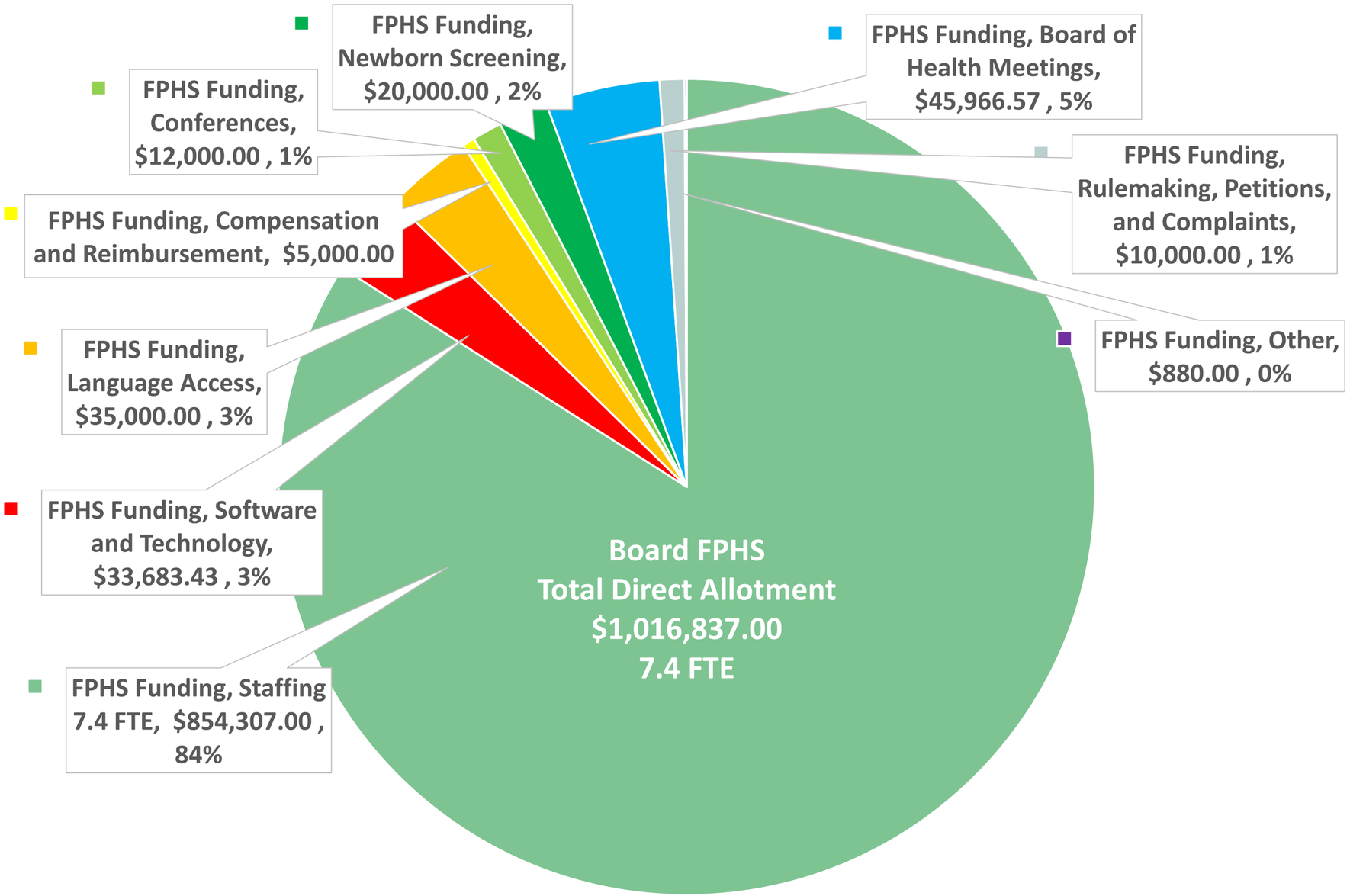
Note: This is showing direct funding only. The formula for total and direct allocations are reflected differently in the budget this year**. FY27 will not have proviso GFS funding**, as this is one-time, for the implementation of ESHB 1946: Local boards of health/Tribes

FY26 General Fund State Overview





FY26 FPHS Overview



Understanding Current Funding

- The Council's recent budget increase was cut in half. This is not sufficient to fully to support all HDC staff and operations.
- FY 2026 FPHS funding investments for SBOH remain unchanged
- FPHS dollars fund 8 staff positions. Because these dollars have not increased, General Fund State covering staff step increases and cost of living adjustments
- For the first time since 2004, the Executive Director position was reviewed and re-banded by the Office of Financial Management
- Historically, the Board has been underspent due to position vacancies and parental leave
- The Board will need additional policy development capacity and operations support to maintain current operations, and to be able to respond to a rapidly changing and uncertain future.

Next Steps

Control Spending:

- Find creative ways to accomplish tasks for low to no cost
- Prioritize state agency spaces for Board meetings
- Limit Board staff and member participation in conferences and training
- Limit travel to essential activities

2026 Decision Package:

- LBOH proviso funding is one-time and limited to FY26
- New, ongoing funding for key staff positions
- Maintenance funding to address past step increases and cost of living adjustments for current staff positions
- Decision Package is contingent on Governor office approval



WASHINGTON STATE BOARD OF HEALTH

THANK YOU

To request this document in an alternate format, please contact the Washington State Board of Health at 360-236-4110, or by email at **wsboh@sboh.wa.gov** | TTY users can dial 711

ACCESSIBILITY AND THE AMERICANS WITH DISABILITIES ACT (ADA)

- The Washington State Board of Health (Board) is committed to providing information and services that are accessible to people with disabilities. We provide reasonable accommodations, and strive to make all our meetings, programs, and activities accessible to all persons, regardless of ability, in accordance with all relevant state and federal laws.
- Our agency, website, and online services follow the Americans with Disabilities (ADA) standards, Section 508 of the Rehabilitation Act of 1973, Washington State Policy 188, and Web Content Accessibility Guidelines (WCAG) 2.0, level AA. We regularly monitor for compliance and invite our users to submit a request if they need additional assistance or would like to notify us of issues to improve accessibility.
- We are committed to providing access to all individuals visiting our agency website, including persons with disabilities. If you cannot access content on our website because of a disability, have questions about content accessibility or would like to report problems accessing information on our website, please call (360) 236-4110 or email wsboh@sboh.wa.gov and describe the following details in your message:
    - The nature of the accessibility needs
    - The URL (web address) of the content you would like to access
    - Your contact information

We will make every effort to provide you the information requested and correct any compliance issues on our website.

School Rule Project Update

