FLUORIDATION PRODUCTS (FLUORIDATED WATERS (TAP OR BOTTLED) AND FLUORIDATION CHEMICAL ADDITIVES) ARE DRUGS

by

Gerald Steel PE
Attorney-at-Law
geraldsteel@yahoo.com

ABSTRACT: This paper presents a legal analysis that demonstrates that fluoridation products (fluoridated waters (tap or bottled) and fluoridation chemical additives) are drugs under the jurisdiction and responsibility of the federal Food and Drug Administrative (FDA) when the intended use is prevention of tooth decay disease.

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TABLE OF CONTENTS

ATTACHMENTS

ABBREVIATIONS

ANDA	Abbreviated New Drug Application
ANSI	American National Standards Institute
CFR	Code of Federal Regulations
DSHEA	Dietary Supplement Health and Education Act
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FDCA	[federal] Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
HHS	Health and Human Services
MCL	Maximum Contaminant Level
MOU	Memorandum of Understanding
NDA	New Drug Application
NRC	National Research Council
NSF	National Sanitation Foundation (now International)
OTC	Over-The-Counter
SDWA	Safe Drinking Water Act
TSCA	Toxic Substances Control Act
USC	United States Code

1. Review of federal drug laws and regulations

a. The 1906 and 1938 Acts of Congress

Drug regulation in the United States began with the Colonies and States adopting isolated laws as early as 1736. (Abigail Alliance for Better Access to Developmental Drugs v. von Eschenbach, 495 F.3d 695, 703-04 (D.C. Cir. 2007).) As early as 1848, the United States began limited drug regulation. (*Id*. at 704.) Congress adopted more comprehensive drug statutes in the Food and Drugs Act of 1906, which prohibited the manufacture of any drug that was "adulterated or misbranded." (*Id*. at 705.) This Act defined "drug" as:

> all medicines and preparations recognized in the United States Pharmacopoeia or National Formulary for internal or external use, and any substance or mixture of substances intended to be used for the cure, mitigation, or prevention of disease of either man or other animals;

and defined "food" as including "articles used for food [and] drink." (Food and Drugs Act of 1906 (emphasis supplied), 34 Stat. 768 (1906).)

Initially, this Act did not regulate false claims of the curative power of a drug but this was changed by Congress in 1912. (Samuels v. United States, 232 F. 536, 545 (8th Cir. 1916).) The 1906 Act, as amended, did not require government approval before a drug was introduced into the market. (United States v. Hiland, 909 F.2d 1114, 1125 (8th Cir. 1990).) This changed with the adoption by Congress of the federal Food, Drug, and Cosmetic Act ("FDCA") of 1938 which required a FDA approved new drug application ("NDA") to demonstrate a drug was safe before entering the market. (Samuels at 545.) No new approvals were required for drugs marketed under the 1906 Act only if their conditions of use remained unchanged. (*Id*.)

b. In 1952, after Congress defined prescription drugs, the FDA announced it would not enforce the FDCA for fluoridated public water

The Durham-Humphrey Amendment of 1951 (65 Stat. 648) for the first time explicitly defined two classes of medications (prescription and over-the-counter ("OTC")). (Christopher v. SmithKline Beecham Corp., 635 F.3d 383, 385 (9th Cir. 2011).) In 1952, in response to this amendment, the FDA adopted a regulation stating:

> (a) The program for fluoridation of public water supplies recommended by the Federal Security Agency, through the Public Health Service, contemplates the controlled addition of fluorine at a level optimum for the prevention of dental caries.
> (b) Public water supplies do not ordinarily come under the provisions of the Federal Food, Drug, and Cosmetic Act. . . .
> (c) The Federal Security Agency will regard water supplies containing fluorine, within the limitations recommended by the Public Health Service, as not actionable under the Federal Food, Drug, and Cosmetic Act.

(Former 21 CFR 3.27 (1952); 17 FR 6732; *infra* at B 23.) This regulation was recodified to former 21 CFR 250.203 in 1975. (40 FR 13996; *infra* at B 24.) It was published, as amended, in 1995. (*Infra* at B 25-26.)

c. In 1996 the FDA reversed its position to not enforce the FDCA regarding fluoridated water after the EPA/FDA MOU was terminated and after Congress adopted the DSHEA that defined minerals as drugs if used to prevent specific diseases

In 1996, the FDA determined that its 1952 regulation was obsolete or no longer necessary and the regulation was revoked. (61 FR 29476; *infra* at B 27.) The revocation of former 21 CFR 250.203 occurred after the federal Environmental Protection Agency ("EPA") announced the "Termination of the Federal Drinking Water Additive Program" effective April 7, 1990. (53 FR 25586-89; CP 142-45; *infra* at B 28-31.) The first and major Term of Agreement

of a 1979 Memorandum of Understanding ("MOU") between FDA and EPA was having EPA develop and operate the federal regulatory drinking water additives program:

> III. Terms of Agreement
>
> A. EPA's responsibilities are as follows:
>
> 1. To establish appropriate regulations, and to take appropriate measures, under the SDWA and/or TSCA, and FIFRA, to control direct additives to drinking water (which encompass any substances purposely added to the water)

(44 FR 42775-78; *infra* at B 33 and at B 38.) Arguably, EPA's Federal Register announcement of termination of its regulatory Federal Drinking Water Additives Program was effective notice to FDA that EPA was terminating the 1979 MOU and EPA was no longer obligated by this MOU to establish and operate a federal regulatory program to control direct additives to drinking water. (44 FR 42776, *infra* at B 33 and B 39 ("This [MOU] shall continue in effect unless . . . terminated by either party upon thirty (30) days advance written notice to the other.”))

The revocation of former 21 CFR 250.203 also occurred after the adoption by Congress of the Dietary Supplement Health and Education Act of 1994 (Pub. L. 103-417; "DSHEA"). This 1994 Act of Congress clarified Congressional intent that mineral additives [including fluoride] are drugs if the intended use is to prevent disease:

> A dietary supplement is deemed to be "food," [21 USC] 321(ff), which is defined in part as "articles used for food or drink for man or other animals," *Id*. § 321(f)(1), **except** when it meets the definition of a "drug," which is defined in part as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals."

(Alliance for Natural Health U.S. v. Sebelius, 714 F.Supp.2d 48, 50 (D.D.C. 2010) (emphasis supplied.)) Under the DSHEA, dietary supplements include minerals. (21 USC 321(ff)(1)(B);

infra at B 42.) **In adopting the DSHEA in 1994, Congress clarified its intent that fluoride minerals when used to prevent disease are drugs under federal law.** (21 USC 321(ff)(postscript), *infra* at B 43.) In 2000, the FDA Commissioner concurs.[1] (*Infra* at B 44.)

d. The 1962 Amendments to the 1938 Act

The Congress amended the FDCA in 1962 to change the standard for approval of a NDA or abbreviated NDA ("ANDA") from "safe" to "safe and effective" for the intended use. (Samuels at 545.) For drugs with approved NDAs under the 1938 Act to retain these NDAs, they were required to demonstrate they were effective. (*Id*.; Weinberger v. Hynson, Wescott & Dunning, Inc, 412 U.S. 609, 612-15, 93 S.Ct. 2469, 37 L.Ed.2d 207 (1973).)

e. In 1972, the FDA established a new approval process for non-prescription drugs

In 1972, the FDA established a new approval process for non-prescription drugs. (21 CFR Part 330.) This process resulted in the establishment of over-the-counter ("OTC") monographs for various drug classifications including a monograph for anticaries drug products that do not require a prescription. (21 CFR Part 355.) The final rule for the anticaries drug monograph is in 60 FR 52473-510. Amendments to this final rule are in 60 FR 57927, 61 FR 52285-87, 64 FR 13296, and 68 FR 24879-80. This final rule, as amended, provides that all OTC anticaries drug products introduced to the market after April 7, 1997 must comply with general conditions in 21 CFR 330.1 and with anticaries monograph conditions in 21 CFR Part

[1] Congress specifically asked FDA to address the relationship of "fluoride in drinking water and drug(s)." (*Infra* at B 44.) The FDA responded, in part, stating "the Environmental Protection Agency regulates fluoride in the water supply." (*Id*.) But EPA had terminated its water additive program more than ten years earlier. (*Supra* at B 2-3.) So FDA was referring to EPA regulating the Maximum Contaminant Level ("MCL") for fluoride that triggers clean-up under the SDWA and was not referring to regulation of fluoride additives for health care purposes.

355; otherwise a NDA or ANDA is required.

> On or after [April 7, 1997] no OTC drug product that is subject to the monograph and that contains a nonmonograph condition . . . may be initially introduced . . . into interstate commerce unless it is the subject of an approved application or abbreviated application.

(60 FR 52474; 61 FR 52285.) Also, it should be noted that FDA regulations provide that any anticaries drug that includes hydrogen fluoride requires a NDA. (21 CFR 310.545(a)(2) and (b).) Typical specification sheets for water treatment certified Fluorosilicic Acid show a significant portion of the fluoride comes from hydrogen fluoride. (*Infra* at B 47.) Some of the fluoride in water treatment certified Sodium Fluoride also comes from hydrogen fluoride. (*Infra* at B 50.)

2. <u>All drinking waters are drugs when fluoridation chemicals are added with intent to prevent, mitigate and/or prophylactically treat tooth decay disease</u>

a. The FDCA explicitly makes articles drugs when intended for use in the treatment, mitigation and/or prevention of disease

> The term "drug" means
> (A) articles recognized in the official United States Pharmacopoeia . . .; and
> (B) **articles intended for use in the** diagnosis, cure, **mitigation, treatment, or prevention of disease in man** or other animals; and
> (C) articles (other than food) intended to affect the structure of 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). . . .

(21 USC 321(g)(1); *infra* at B 41; emphasis supplied.) The language quoted has not been amended since it was originally adopted in the 1938 Act. (52 Stat. 1041.)

b. Fluoridated drinking waters (bottled or tap (from public water systems)), and fluoridation chemical additives (whether or not certified under NSF/ANSI Standard 60) are drugs under 21 USC 321(g)(1) when the intended use is to aid in the prevention, mitigation and/or prophylactic treatment of dental caries disease (tooth decay, cavities)

Based on 21 USC 321(g)(1)(B) when fluoridated drinking water is intended to aid in the prevention, mitigation and/or prophylactic treatment of dental caries disease (tooth decay, cavities) it is a drug under the FDCA. **There is nothing in the FDCA that would suggest otherwise and HHS and FDA have not made the claim that there is.** Similarly, based on 21 USC 321(g)(1)(B) fluoridation chemical additives that are intended to aid in the prevention, mitigation and/or prophylactic treatment of dental caries disease are drugs under the FDCA. When fluoridation chemical additives are intended for use as a component of fluoridated drinking water, then these fluoridation chemical additives are also drugs under 21 USC 321(g)(1)(D). There is no provision in the FDCA that would cause either fluoridated tap water or fluoridated bottled water to not be considered a drug when the intended use is to aid in the prevention, mitigation and/or prophylactic treatment of dental caries disease (tooth decay, cavities).

c. It should be presumed that the intended use of fluoridation chemical additives and fluoridated waters (bottled or tap) using such additives is to aid in the prevention, mitigation and/or prophylactic treatment of dental caries disease (tooth decay, cavities)

Today, in almost every state, water fluoridation chemical additives are required to be certified to ANSI/NSF Standard 60. For example, in Washington State:

> Any treatment chemicals, with the exception of commercially retailed hypochlorite compounds such as unscented Clorox, Purex, etc., added to water intended for potable use must comply with ANSI/NSF Standard 60. The maximum application dosage recommendation for the product certified by the ANSI/NSF Standard 60 shall not be exceeded in practice.

WAC 246-290-220(3). NSF, an author of ANSI/NSF Standard 60, states in its 2008 NSF Fact Sheet on Fluoridation Chemicals:

> Water fluoridation . . . . Fluoride is added to water for the public health benefit of preventing and reducing tooth decay

(*Infra* at B 73.) In 2011, HHS confirmed its belief that:

> Community water fluoridation is the most cost-effective method of delivering fluoride for the prevention of tooth decay.

(76 FR 2386; *infra* at B 51.)

The FDA has concluded that the intended use is implied for fluoride additives to prevent tooth decay. The FDA finds that intended use "may be shown by the circumstances surrounding the distribution of the article." (21 CFR 801.4.) The FDA states:

> in some instances, the mere presence of certain therapeutically active ingredients could make a product a drug even in the absence of drug claims. In these cases, the intended use would be implied because of the known or recognized drug effects of the ingredient (e.g. fluoride in a dentifrice).

(59 FR 6088.) The intended use of added fluoride in drinking water is also implied and should be presumed. The FDA's interpretation of "intent" is entitled to "considerable deference." (Young v. Community Nutrition Institute, 476 U.S. 974, 981, 106 S.Ct. 2360, 90 L.Ed.2d 959 (1986).) The Washington State Board of Health states,

> The Board considers it self-evident that the purpose of water fluoridation is to help prevent tooth decay.

(*Infra* at B 52.)

The CDC states, "Tooth decay (dental caries) is an infectious, multifactorial disease." (Infra at B 54.) The FDA defines "dental caries" as "A disease of calcified tissues of teeth characterized by demineralization of the inorganic portion and destruction of the organic matrix" and defines "anticaries drug" as "A drug that aids in the prevention and prophylactic treatment of

dental cavities (decay, caries). (21 CFR 355.3(c) and (d).)

d. The language in 21 USC 321(g)(1)(B) defining drugs must be interpreted "as broad as its literal language indicates"

As early as 1916, the federal Supreme Court concurred that products that were otherwise defined as "foods" would be "drugs" under the federal statute[2] when labeling for the substance includes statements of therapeutic (including preventative) effect. (Seven Cases v. United States, 239 U.S. 510, 513-14, 36 S.Ct. 190, 60 L.Ed. 411 (1916).)

After the 1938 Act was adopted, the federal Supreme Court again concurred that "food products" will be "drugs" based on intended use and "labeling." (Kordel v. United States, 335 U.S. 345, 346, 69 S.Ct. 106, 93 L.Ed. 52 (1948).) In 1969, the federal Supreme Court, in finding a product was a drug, explained:

> Congress intended to define "drug" [in 21 USC 321(g)(1)(B)] far more broadly than does the medical profession. . . . The word "drug" is a term of art for the purposes of the Act, encompassing far more than the strict medical definition of that word.

(United States v. An Article of Drug . . . Bacto-Unidisk, 394 U.S. 784, 793, 89 S.Ct. 1410, 22 L.Ed.2d 726 (1969).) The Bacto-Unidisk Court continued:

> Congress fully intended that the Act's coverage be **as broad as its literal language indicates** - and, equally clear, broader than any strict medical definition might otherwise allow. . . . **the Food, Drug, and Cosmetic Act is to be given a liberal construction consistent with the Act's overriding purpose to protect the public health.**

(*Id*. at 798; emphasis supplied.) The Bacto-Unidisk Court finally directed,

> we must take care not to narrow the coverage of a statute short of the point where Congress indicated it should extend.

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[2] The relevant portion of the federal statute are quoted *supra* at B 1.

(*Id*. at 801.)

In the construction of federal statutes, "the decisions of the Supreme Court of the United States are binding" upon all. (Beezer v. City of Seattle, 62 Wn.2d 569, 573, 383 P.2d 895 (1963).) Therefore, HHS and FDA and every court is required to construe the definition of drug as "articles intended for use in the . . . prevention of disease" as "broad as its literal language indicates." (*Supra*.)

e. Foods must be regulated as drugs if the "intended use" is to prevent disease

Interpretation of federal statutes by other federal courts are entitled to great weight. (Beezer at 573.) A long line of federal court cases has found that articles normally regulated as "foods" will be regulated as "drugs" if the intended use is to treat or prevent a disease:

> The word "drug" is defined in 21 U.S.C. s 321(g)(1)(B) to include:
>
> articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals . . .
>
> Thus, it is the intended use of an article which determines whether or not it is a "drug," and even the most commonly ingested foods and liquids are "drugs" within the meaning of the [FDCA] if their intended use falls within the definition of s 321(g)(1)(B).

Gadler v. United States, 425 F.Supp. 244, 246-47 (D.Minn. 1977); *see* Nutrilab, Inc. v. Schweiker, 713 F.2d 335, 336 (7th Cir. 1983); *see also* Bradley v. United States, 264 F.79 (5th Cir., 1920) where the court specifically found "mineral water" to be a "drug" when it is intended to treat disease.

In the determination of whether fluoridation products (fluoridated waters (tap or bottled) and fluoridation chemical additives) are drugs,

> the only question under the [FDCA] is whether the intended use of the product is to prevent disease, not whether the product actually prevents disease.

(United States v. Bowen, 172 F.3d 682, 686 (9th Cir. 1999).) Intent "may be derived or inferred from [any] relevant source." (National Nutritional Foods Ass'n v. Mathews, 557 F.2d 325, 334 (2nd Cir. 1977).) As discussed previously, it should be presumed that the "intended use" of fluoridation products is to prevent dental caries (tooth decay) disease. (*Supra* at B 6-8.)

f. The DSHEA further clarifies the intent of Congress that fluorides, which are minerals, that are added to drinking water to prevent the disease of dental caries, are drugs

Perhaps partly in response to the FDA's refusal to enforce the FDCA for fluoridated water supplies (*supra* at B 2), Congress adopted the DSHEA in 1994, with explicit statutory language that made fluoride a drug when used with intent to prevent disease. Fluoride, being a mineral, is a dietary supplement under DSHEA. (21 USC 321(ff)(1)(B); *infra* at B 42.) Minerals are normally regulated as foods except when they are drugs. (21 USC 321(ff)(postscript) ("**except** for purposes of [21 USC 321(g)(1) defining drugs] a dietary supplement shall be deemed to be a food;") *infra* at B 43 (emphasis supplied).)

g. Congress did not intend to exempt public water or water additives from the reach of federal drug laws

In 1974, Congress passed the Safe Drinking Water Act ("SDWA"). (88 Stat. 1661; codified at 42 USC 300f et seq.) The SDWA empowered the EPA to set standards for the control of contaminants in drinking water. (42 USC 300g-1(b); *see* In re Groundwater Cases, 154 Cal.App.4th 659, 677 (2007).) The SDWA authorizes EPA to adopt national primary drinking water regulations applicable to "public water systems." (42 USC 300f(1); *see* 42 USC

300f(4)(A).) Under the SDWA, national primary drinking water regulations identify contaminants that have adverse effects on human health and specify a maximum contaminant level ("MCL") for such contaminants. (42 USC 300f(1).) Pursuant to its authority under the SDWA, the EPA has since established MCLs for a wide variety of contaminants. (*See* 40 CFR Pt. 141 for substantive regulations, Pt. 142 for implementation regulations, and Pt. 143 for national secondary drinking water regulations that are not enforceable.) The fluoride MCL is 4.0 mg/l (four milligrams per liter which is 4 parts per million (ppm)). (40 CFR 141.62(b)(1).)

But there is no SDWA statutory provision or implementing regulation that addresses or sets standards for fluoridation chemical additives.[3] (SDWA; 40 CFR Part 141 et seq.) Therefore, there is no possible statutory conflict where Congress intended the SDWA to interfere with the FDCA or FDA authority to regulate drugs. If Congress wanted to exempt public drinking water from the definition of drugs in 21 USC 321(g)(1)(B) it certainly had the knowledge of how to do it (it had previously exempted "food" from subsection (1)(C)) and it certainly had the opportunity to do it in any one of the more than 20 significant amendments made to the FDCA since 1980. (*Infra* at B 56-57.) The SDWA did not explicitly or implicitly repeal any drug provision of the FDCA or any drug authority of the FDA.

h. Arguably, the 1979 EPA/FDA MOU has been terminated but never did restrict FDA authority over drugs

i. <u>The 1979 MOU</u>

In 1979, EPA and FDA entered into an MOU where FDA agreed not to enforce its food

[3] There is a SDWA statutory provision that directs the EPA to keep away from regulating drugs. (42 USC 300g-1(b)(11) ("No national primary drinking water regulation may require the addition of any substance for preventive health care purposes unrelated to contamination of drinking water."))

authority over public drinking water in exchange for EPA creating a federal regulatory drinking water additives program. (*Infra* at B 32-39.) In the FDCA, Congress gave FDA authority to regulate foods to ensure they are "safe" (21 USC 393(b)(2)(A)) and drugs to ensure they are "safe and effective" (21 USC 393(b)(2)(B)). Normally for drinking water, only food regulations would be applicable and prior to 1979, the FDA generally regulated drinking water as a food. (*Infra* at B 32 and B 37.) But after passage of the SDWA, EPA and FDA were concerned that FDA's "food" authority and EPA's "public drinking water" authority might result in "duplicative and inconsistent regulations" so they entered an MOU. (*Supra* at B 2-3, *Infra* at B 32.) In the MOU, FDA agreed not to use its "food" authority to regulate public drinking water, based on a commitment that EPA would adopt federal regulations to control additives in public drinking water. (*Supra* at B 2-3, *Infra* at B 32-33.)

There is no mention in the MOU that FDA would, or could, give up its "drug" authority over public drinking water and public drinking water additives. (Infra at B 32-39.) Congress required "drugs" to be "effective" (21 USC 393(b)(2)(B)) and Congress never gave EPA authority to regulate drug effectiveness. The MOU inartfully states:

> [EPA and FDA] have determined that the passage of the SDWA in 1974 implicitly repealed FDA's authority under the [FDCA] over water used for drinking water purposes.

(*Infra* at B 32.) Read in context with the other provisions of the MOU this can only possibly be true with respect to FDA's "food" authority and cannot be true with respect to FDA's "drug" authority. (*Infra* at B 32-34; *See* Board of Governors of the Federal Reserve System, 474 U.S. 361, 368, 106 S.Ct. 681, 88 L.Ed.2d 691 (1986) ("agency interpretation" cannot "alter the clearly expressed intent of Congress."))

In a subsequent section, the MOU states:

> [EPA and FDA] agreed that the Safe Drinking Water Act's passage in 1974 implicitly repealed FDA's jurisdiction over **drinking water as a "food"** under the [FDCA].

(*Infra* at B 33; emphasis supplied.) Thus the MOU itself clarifies that the MOU only was intended to address FDA's regulations regarding "food." The MOU also inartfully states:

> Under the agreement, EPA now retains exclusive jurisdiction over drinking water served by public water supplies, including any additives in such water.

(*Infra* at B 33.) In context of the whole agreement, EPA does not have exclusive jurisdiction when public drinking waters, and public drinking water additives, are "drugs" because Congress has given exclusive jurisdiction over drugs to the FDA. (21 USC 393(b)(2)(B); FDA v. Brown & Williamson Tobacco Corp., 529 U.S. 120, 126, 120 S.Ct. 1291, 146 L.Ed.2d 121 (2000).) Congress has clearly defined "drugs" in 21 USC 321(g)(1). Further EPA claims no authority that would give it jurisdiction over the determination of "effectiveness" of drugs. (*Infra* at B 32-35.)

ii. Arguably, the 1979 MOU is terminated

In 1988, EPA published in the Federal Register a "Notice"that it was terminating EPA's commitment to FDA to create a federal regulatory drinking water additives program. (53 FR 25586-89; *infra* at B 28-31.) In this 1988 Notice, EPA admits that it "does not currently regulate the levels of additives in drinking water." (*Infra* at B 28.) EPA explained that the "SDWA does not require EPA to control the use of specific additives in drinking water." (*Infra* at B 28.) It states,

> Resource constraints and the need to implement mandatory provisions of the SDWA precluded the Agency from implementing the comprehensive program originally

envisioned . . .

(*Infra* at B 29.) The Notice describes how EPA was cooperating with a private third-party organization to have that organization take over the development and monitoring of standards for public drinking water additives and explained that it would be "up to the States and utilities to determine the suitability of any 'third-party' certification." (*Infra* at B 28-30.) Then it announced that effective April 7, 1990, it would withdraw all EPA and predecessor agency lists of acceptable water additive products and all EPA and predecessor agency advisory opinions on drinking water additives. (*Infra* at B 31.) EPA stated that "Discontinuance of the additives program at EPA does not relieve the Agency of its statutory responsibilities." (*Infra* at B 31.)

Arguably, EPA's Federal Register published Notice that it was terminating its commitment to FDA to create a regulatory federal drinking water additives program was effective notice to FDA that EPA was exercising its option to terminate the MOU. *(Supra* at B 2-3.) Thus, arguably, the 1979 MOU was terminated by 1990 and EPA removed the cloud over FDA's "food" jurisdiction regarding public fluoridated water. FDA never lost "drug" jurisdiction over fluoridated water, but its policy, that it would not enforce this jurisdiction, remained in effect from 1952 to 1996. (*Supra* at B 2-3.)

i. The intent of Congress clearly establishes that water fluoridation products are drugs under the FDCA

In 1916, the federal Supreme Court concurred that Congress in adopting the 1906 Act directed that food be regulated as a drug when therapeutic (including preventative) effects are intended. (*Supra* at B 8.) In the 1938 Act, Congress significantly broadened, instead of limited, the definition of drugs. (*Compare supra* at B 1 and B 5.) In 1948, the federal Supreme Court

again concurred "food products" will be "drugs" depending on intent and "labeling." (*Supra* at B 8.)

In 1952, the FDA stated it would not enforce the FDCA for fluoride added to public water supplies. (*Supra* at B 2.) In 1969, the federal Supreme Court ruled that the FDCA definition of drugs is "as broad as its literal language indicates." (*Supra* at B 8-9.) In 1994, the Congress again specifically clarified that minerals will be drugs if they fall within the broad definition of drugs. (*Supra* at B 3-4 and 10.) In 1996, the FDA revoked its policy that it would not enforce the FDCA for fluoride added to public water supplies. (*Supra* at B 2.)

Every department and agency and court is bound by the intent of Congress as explained by the federal Supreme Court. (*Supra* at B 9.) Therefore, the FDA should find that water fluoridation products (fluoridated waters (tap or bottled) and fluoridation chemical additives) are drugs under federal law and regulation when the intended use is to aid in the prevention, mitigation, and/or prophylactic treatment of dental caries disease (tooth decay, cavities). And based on the history of fluoridation, it should be presumed that this is the intended use of water fluoridation products. (*Supra* at B 9-10.)

3. HHS, acting through the FDA, is responsible for regulating the addition of fluoride to public drinking water

_____ Despite the Federal Supreme Court ruling in Bacto-Unidisk (*supra* at B 8-9), HHS and FDA appear to now argue that certain fluoridation products (fluoridated public waters and fluoridation chemical additives) are not drugs. It is uncontested by HHS and FDA that these fluoridation products are articles intended to prevent dental caries disease in man. (*Supra* at B 6-8.) Under Bacto-Unidisk and other federal court rulings (*supra* at B 7 to B 9), these fluoridation

products are therefore within the definition of a "drug" in 21 USC 321(g)(1)(B).

However, HHS and FDA interpret the Safe Drinking Water Act of 1974 (SDWA) as removing HHS and FDA jurisdiction over these fluoridation products:

> *Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the FD&C Act. Instead, Congress intended that the U.S. Environmental Protection Agency (EPA) regulate fluoride in public drinking water as a potential contaminant under the Safe Drinking Water Act of 1974 (SDWA).*

(*Infra* at B 59 and B 66: B 58 to B 62 is a November 21, 2014 letter from HHS Principal Deputy Assistant Secretary for Health Dr. Wanda Jones to Ms. McEtheney; B 63 to B 68 is a December 23, 2013 Request for Review to Jill Warner, FDA Associate Commissioner for Special Medical Programs from Gerald Steel (FDA has not yet responded to this Request for Review).)

HHS and FDA argue that the SDWA provides:

> that within the limits thus set by EPA, state and local governments be permitted, but not required, to fluoridate public drinking water to help prevent dental caries.

(*Infra* at B 59 and B 66.) Thus, HHS and FDA argue that under their interpretation of the SDWA, FDA has no responsibility to regulate such fluoridation products that are articles that meet the definition of a drug in 21 USC 321(g)(1)(B).

The fundamental problem with this HHS and FDA interpretation of the SDWA is that it is in conflict with the EPA interpretation of the SDWA. The SDWA gives administrative authority to the EPA. (42 USC 300f(7 and 8).) Along with administrative authority comes the sole agency power to interpret the Act. Chevron USA v. NRDC, 467 U.S. 837, 842-45, 104 S.Ct. 2778, 81 L.Ed.2d 694 (1984).

Steven M. Neugeboren is the Associate General Counsel in charge of the Water Law Office of the EPA. The Water Law Office is responsible for interpreting the SDWA.[4] Mr. Neugeboren states:

> Under the Safe Drinking Water Act (SDWA), EPA is the lead federal agency with responsibility to regulate the safety of public water supplies. EPA does not have responsibility for substances added to water solely for preventative health care purposes, such as fluoride, other than [to meet maximum contaminant limits.] The Department of Health and Human Services (HHS), acting through the FDA, remains responsible for regulating the addition of drugs to water supplies for health care purposes.

(*Infra* at 69-70 - February 14, 2013 letter written on behalf of EPA Administrator Lisa Jackson to Gerald Steel.) Therefore the EPA's interpretation of the SDWA is that this Act does not affect the responsibility of the FDA "for regulating the addition of drugs to water supplies for health care purposes." Therefore HHS and FDA misinterpret Congressional intent when they state:

> Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the FD&C Act.

(Infra at B 59 and B 66.)

HHS and FDA are correct that the SDWA does give EPA lead responsibility for regulating the safety of public water supplies to protect against adverse health effects. Except for authorizing regulation of the maximum contaminant level for fluorides, the SDWA does not address state and local governments fluoridating public drinking water to help prevent dental caries. But the state and local governments which fluoridate must comply with all applicable laws and regulations including federal drug laws in the FDCA, state drug and fluoridation laws,

[4] http://www2.epa.gov/aboutepa/about-office-general-counsel-ogc#water

federal drug regulations, and state drug and fluoridation regulations. The EPA has determined that state fluoridation regulations are not related to the SDWA. (*Infra* at B 71-72 - November 17, 2011 letter written on behalf of EPA Region 10 Administrator to Gerald Steel.)

Under this analysis and the interpretations of the SDWA by the EPA: HHS and FDA should find that fluoridation products are drugs when they meet the definition of a drug in 21 USC 321(g)(1)(B). HHS, acting through the FDA, has responsibility to regulate these drugs to ensure that they are safe and effective.

4. FDA should request registration of all water fluoridation products as drugs pursuant to 21 CFR Part 207

It is requested that FDA request registration of all water fluoridation products as drugs pursuant to 21 CFR Part 207. In most states, lists of public water purveyors making fluoridated waters are available from State Health Departments. In most states, fluoridation chemical additives must be certified to meet ANSI/NSF Standard 60. (*Supra* at B 6.) There are only three organizations that certify products to ANSI/NSF Standard 60 and their web addresses are www.nsf.org/, www.ul.com/eph/, and www.wqa.org/. These organizations can be contacted to get current lists of ANSI/NSF Standard 60 certified fluoridation chemical additive products and manufacturers.

To facilitate determination of the legal drug status of these fluoridation products, it is requested that FDA request for each fluoridation product, for each year the product was marketed or proposed for future use, a copy of all certificates of analysis and product labeling (both on any packaging and from any other documents (electronic, print, or otherwise) describing the product or describing the purpose of using fluoride additives, or describing the conditions of use that are

recommended or suggested.) For water purveyors, the documents describing the purpose of fluoride additives, likely would include documents associated with the decision to begin fluoridation and documents, including materials sent to customers, that later describe on-going reasons for fluoridation. Because certification to ANSI/NSF Standard 60 began around 1990, it is expected that fluoridation chemical additive labeling was changed around that time to declare certification. It is likely that all fluoridation product manufactures will be required to get approved new drug applications or approved abbreviated new drug applications.

5. FDA should find that fluoridation products are not "safe and effective"

Once it accepts jurisdiction, FDA should find that fluoridation products are not safe and effective as drugs. While this is a subject that will only be addressed after HHS and FDA accept drug jurisdiction over fluoridation products, it is useful to point out the harms that HHS and FDA are allowing to occur because they have not accepted drug jurisdiction over fluoridation products.

An important overview was provided in the York Review in 2000 (M. McDonagh, P. Whiting, M. Bradley, et al., "A Systematic Review of Water Fluoridation," NHS Centre for Reviews and Dissemination, The University of York, Report 18 (2000) which is available at: (http://www.york.ac.uk/inst/crd/CRD_Reports/crdreport18.pdf). The potential harms explored by the York Review include dental fluorosis, hip fracture, other bone fractures, cancer, Down's syndrome, mortality, senile dementia, goitre, lowered IQ, hypersensitivity, and skeletal fluorosis. (York Review at 52, 54, 59-60.) The York Review concludes that except for dental fluorosis, no "confident statements" can be made regarding these "potential harms." (York Review at page xiv.) In other words, these other "potential harms" could not be ruled out by the available scientific literature.

a. Dental fluorosis is an out-of-control harm of water fluoridation

There is scientific consensus that fluoridated water causes dental fluorosis. HHS reported that 41% of people who were 12 to 15 years old in 1999 to 2004 had dental fluorosis with this dental fluorosis being moderate or severe for 3.6% of these people (one in twenty eight people). (76 FR 2385.) Even if water fluoridation is reduced to 0.7 mg/l fluoride as HHS now recommends, the number of people with dental fluorosis is likely to increase because in 1992 when these people were 0 to 8 years old, only 56% of the people in the United States received fluoridated water. Today a much higher percentage of people receive fluoridated water.

b. The FDA has already concluded that fluoride OTC products should not be swallowed except under professional supervision

The FDA has already concluded that fluoride OTC anti-cavity products should not be swallowed except under professional supervision. (21 CFR Part 355.) Fluoridation chemical additives are intended to be mixed with water and swallowed by everyone. At a minimum, fluoridated water is harmful to infants and children under 6. Warnings are required for OTC products to avoid swallowing by infants and even children under six. (21 CFR 355.50.) Bottled water regulations do not even allow a health claim for fluoridated water marketed to infants. (www.fda.gov/food/ingredientspackaginglabeling/labelingnutrition/ucm073602.htm)

c. York Review studies repeatedly show that artificial water fluoridation increases risk of hip fracture in people 65+ years old

The York Review was limited to review of human epidemiological studies of water fluoridation (around 1 mg/l fluoride). Over 3,200 primary studies were identified but only 9 studies met relevance criteria and measured risk of hip fracture for people 65+ years old in fluoridated areas compared to the risk in unfluoridated areas. (York Review at 10 and 48.) For

these 9 studies, there were only 4 analyses that produced statistically significant data (i.e. the relative risk of 1.0 was not in the 95% Confidence Interval). Each of these statistically significant analyses show an increased risk of hip fracture for people 65+ years old living in fluoridated areas. The studies are identified in the York Review at page 48 as:

Author (Year)	Sex	Relative Risk	95% Confidence Interval
Jacqmin-Gadda (1998)	Both	2.43	(1.1, 5.3)
Danielson (1992)	Women	1.27	(1.1, 1.5)
Jacobsen (1992)	Women	1.08	(1.06, 1.10)
Jacobsen (1992)	Men	1.17	(1.13, 1.22)

Relative Risk is defined as the risk of an adverse effect with exposure to a treatment (here fluoridated water) relative to risks for those who do not receive the treatment. (York Review at 99.) A ratio of 1.0 indicates no increased risk over receiving no treatment. (*Id*.) A ratio greater than 1.0 indicates the risk is higher in the group that did receive the treatment. (*Id*.) A ratio less than 1.0 indicates the risk of the adverse effect is higher in the group that did not receive treatment. (*Id*.) A Relative Risk of 1.27 means that there is a 27% higher risk of hip fractures when living in a fluoridated area (for 65+ year old women in the Danielson (1992) analysis).

Hip fracture for people 65+ years old is a significant health impact in the United States. "About 300,000 Americans are hospitalized for a hip fracture every year." (Connett (2010) at page 173.) The Irish Forum (2002) (Forum on Fluoridation (Dublin, Ireland: Stationery Office, 2002) online at http://fluoridealert.org/re/fluoridation.forum.2002.pdf found that "Fracture of the hip is a major cause of morbidity and mortality [disease and death] in persons 65 years of age and older."

> Aside from the fact that one in five patients die within 6 months of the fracture occurring, hip fractures lead to serious disability. Many basic functions such as dressing, climbing stairs, walking and transferring are markedly interfered with following a fracture. This can result in loss of both confidence and independence and an increased risk of development of medical complications.

(Irish Forum (2002) at 121.)

d. Fifty human studies agree that higher fluoride exposure is associated with a mental health impact that lowers IQ levels in children

Lowered IQ in persons who drink fluoridated water as infants and children is a significant mental health concern. The National Research Council (2006) states, "It is apparent that fluorides have the ability to interfere with the functions of the brain." (NRC, Fluoride in Drinking Water - A Scientific Review of EPA's Standards (Washington D.C.; The National Academies Press, 2006.) As of September, 2016, 50 of 57 human studies found elevated fluoride exposure is associated with reduced IQ and 45 animal studies have found fluoride exposure impairs the learning and/or memory capacity of animals. (http://fluoridealert.org/studies/brain01/)

> The lowest level at which IQ has been lowered (with borderline iodine deficiency) was at 0.88 ppm [fluoride in drinking water] (Lin et al., 1991) or at 1.26 ppm (without iodine as a complicating factor). It is very clear that there is no margin of safety to protect all children drinking water in the range 0.7 to 1.2 ppm.

Dec. 12, 2014 email from Paul Connett, PhD., then Director, Fluoride Action Network.

e. Drinking fluoridated water increases risk of hypothyroidism disorder

A large observational study was published in the online Journal Of Epidemiology and Community Health, a British Medical Journal (BMJ) publication, on February 24, 2015 that found rates of diagnosed hypothyroidism (underactive thyroid) were at least 30% higher in areas with artificial fluoridation. (Peckham (2015) -J Epidemiol Community Health doi:10.1136/jech-2014-204971.) The study states that thyroid dysfunction is a common endocrine disorder. The National

Research Council ((2006) at 223 called fluoride an endocrine disrupter and at 218 expresses concern about "the inverse correlation between asymptomatic hypothyroidism in pregnant mothers and the IQ of the offspring."

f. Boys drinking fluoridated water when they are 6 to 8 years old have a five to seven-fold greater risk of contracting bone cancer by the age of twenty

Regarding cancer, an unrefuted published primary study, Bassin (2006) (Bassin E. B. et al., "Age-specific Fluoride Exposure in Drinking Water and Osteosarcoma (United States)," Cancer Causes and Control 17, no. 4 (May 2006) 421-28) reports that boys who drink fluoridated water when they are 6 to 8 years old will have a five- to sevenfold greater risk of contracting osteosarcoma (bone cancer) by the age of twenty. This is a deadly disease. This result was first suggested by Perry Cohn in 1992. (*See* Connett (2010) at pages 187-94.) The twofold increase in cortical bone defects in the fluoridated city in the Kingston-Newburgh study (*supra* at B 20.) was described in 1955 and again in 1977 as being "strikingly similar to that of osteogenic sarcoma [now called osteosarcoma]." (*See* Connett (2010) at page 181-94.)

6. <u>FDA has correctly determined that fluoridated bottled water is a drug when there is a claim that "this drinking water is intended for use in the prevention of tooth decay disease"</u>

In a September 23, 2015 letter (B 74-75 hereto), the FDA found that fluoridated bottled water with 0.7 mg/l fluoride would be a drug if the claim is made that "this drinking water is intended for use in the prevention of tooth decay disease." In fact, fluoridated bottled water with this claim would be an "anticaries drug" as that term is defined by the FDA in 21 CFR 355.3(c) and (d). (*Supra* at B 7-8.) Such fluoridated bottled waters when introduced after April 7, 1997 would be required to have an approved New Drug Application (NDA) or Abbreviated NDA

(ANDA) because they would not be able to meet requirements of 21 CFR Part 355 which do not allow anticaries drugs to be swallowed without professional supervision. (*See supra* at B 4-5.) Under current law, it would be illegal to distribute such fluoridated bottled water in interstate commerce without an approved NDA or ANDA. Because such fluoridated bottled waters would be drugs, the fluoridation chemical additives, which are a component of such fluoridated bottled waters, would also be drugs. (21 USC 321(g)(1)(D).)

7. FDA must now find that fluoridated tap water is a drug when there is a claim that "this drinking water is intended for use in the prevention of tooth decay disease"

FDA must now find that fluoridated tap water is a drug when there is a claim that this drinking water is intended for use in the prevention of tooth decay disease. The FDA must also find that the fluoridation chemical additives, which are a component of such fluoridated tap waters, are also drugs. (USC 321(g)(1)(D).) The FDCA allows no distinction between fluoridated waters with the same contents whether they are served as drinking water either from a bottle or from a tap. Both are anticaries drugs under the FDCA if the drinking water is intended for use in the prevention of tooth decay disease. More generally, fluoridated drinking waters are anticaries drugs if the intended use is to aid "in the prevention and prophylactic treatment of dental cavities (decay, caries)." (21 CFR 355(3)(c).)

Today, as fluoridated water purveyors modify their fluoridated waters to meet the latest HHS recommendation to add fluoride to get 0.7 mg/l fluoride in the finished water, these water purveyors are making a new drug and are subject to new drug requirements for an approved NDA or ANDA and subject to the FDA requirements to show that their unique products are safe and effective.

The FDA can no longer rely on its prior reasoning (*Infra* at B 59 and B 66) that the intent of the SDWA was to eliminate FDA authority and responsibility under the FDCA to regulate substances that qualify as anticaries drugs under the USC and CFR. EPA is the agency with final agency authority to interpret the SDWA, and EPA interprets the SDWA to not remove the authority of HHS, acting through the FDA, regarding "regulating the addition of drugs to water supplies for health care purposes." (*Infra* at B 69.)

So while it is true that state and local governments may be permitted to fluoridate drinking waters to help prevent dental caries, they must do so in compliance with local, state, and federal laws and regulations which include federal requirements to consider such fluoridated waters to be drugs if the drinking waters are "intended for use in the prevention of tooth decay disease" or if the drinking waters otherwise meet the definition of drugs in section 201(g)(1) of the FDCA (21 USC 321(g)(1)). FDA, acting on behalf of HHS, has the authority and responsibility to regulate drugs by implementing the applicable federal laws and regulations and by adopting regulations when necessary to fulfill its responsibilities.

It is time for the FDA to be responsible and to require fluoridation products (fluoridated waters (tap or bottled) and fluoridation chemical additives) to be federally regulated as drugs when the intended use is prevention of tooth decay disease.

customs Form 4449 showing the name of the airport, date and time of arrival, date and time of departure and purpose of the visit. The permit shall be surrendered to the collector of customs at the port of final clearance for a foreign destination, who shall satisfy himself prior to the issuance of clearance that the aircraft received proper customs treatment while in this country. The permit shall then be returned to the collector of customs at the port of issue.

(2) A copy of the permit shall be retained by the collector at the port where issued. If within 60 days after the issuance of such permit the said collector does not receive a report of the outward clearance of the aircraft covered thereby, the matter shall be reported to the supervising customs agent for investigation.

(3) Civil aircraft registered in the United States arriving from a foreign country with passengers carried for hire or merchandise, after proper customs treatment of their cargo (passengers carried for hire or merchandise), may be allowed to proceed upon their identity being established.

This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(R. S. 161, sec. 23, 5 Stat. 892, as amended, sec. 24, 43 Stat. 166, R. S. 251, secs. 624, 644, 46 Stat. 759, 761, secs. 201, 367, 58 Stat. 683, 706, sec. 7, 44 Stat. 57, as amended; 5 U. S. C. 22, 8 U. S. C. 102, 222, 19 U. S. C. 66, 1624, 1644, 42 U. S. C. 202, 270, 49 U. S. C. 177)

[SEAL] D. A. STRUBINGER,
Acting Commissioner of Customs.
JOHN S. GRAHAM,
Acting Secretary of the Treasury.
W. P. DEARING,
Acting Surgeon General,
U. S. Public Health Service.
JOHN L. THURSTON,
Acting Federal Security Administrator.
PHILIP B. PERLMAN,
Acting Attorney General.

JULY 17, 1952.

[F. R. Doc. 52-8054; Filed, July 22, 1952; 8:55 a. m.]

[T. D. 53046]

PART 10—ARTICLES CONDITIONALLY FREE, SUBJECT TO A REDUCED RATE, ETC.

SUPPLIES FOR VESSELS OF WAR

The Department of State has furnished the Treasury Department an up-to-date list of countries which permit the withdrawal of supplies free of duty and tax by vessels of war of the United States while in ports of those countries. Therefore, § 10.59 (d), Customs Regulations of 1943 (19 CFR 10.59 (d)), containing a list of countries whose vessels of war shall be accorded the privilege of withdrawing supplies free of customs duties and internal-revenue tax while in ports of the United States, as provided for in section 309 (a), Tariff Act of 1930, as amended, is further amended to read as follows:

§ 10.59 *Exemption from customs duties and internal revenue tax.* * * *

(d) The privilege shall be accorded to vessels of war of the following countries:

Argentina.
Australia.
Belgium.
Brazil.
Canada.
Chile.
Colombia.
Cuba.
Denmark.
The Dominican Republic.
Ethiopia.
Finland.
France.
Great Britain.
Greece.
Haiti.
India.
Ireland.
Mexico.
The Netherlands.
New Zealand.
Nicaragua.
Norway.
Panama.
The Philippines.
El Salvador.
Spain.
Sweden.
Thailand.
Turkey.
Union of South Africa.
Uruguay.
Venezuela.

(Sec. 5, 52 Stat. 1080; 19 U. S. C. 1309)

[SEAL] FRANK DOW,
Commissioner of Customs.

Approved: July 16, 1952.

JOHN S. GRAHAM,
Acting Secretary of the Treasury.

[F. R. Doc. 52-8025; Filed, July 22, 1952; 8:48 a. m.]

TITLE 21—FOOD AND DRUGS

Chapter I—Food and Drug Administration, Federal Security Agency

PART 3—STATEMENTS OF GENERAL POLICY OR INTERPRETATION

FLUORIDATED WATER AND PROCESSED FOODS CONTAINING FLUORIDATED WATER

Pursuant to section 3 of the Administrative Procedure Act (60 Stat. 237, 238; 5 U. S. C. 1002), the following statement of policy is issued:

§ 3.27 *Status of fluoridated water and foods prepared with fluoridated water under the Federal Food, Drug, and Cosmetic Act.* (a) The program for fluoridation of public water supplies recommended by the Federal Security Agency, through the Public Health Service, contemplates the controlled addition of fluorine at a level optimum for the prevention of dental caries.

(b) Public water supplies do not ordinarily come under the provisions of the Federal Food, Drug, and Cosmetic Act. Nevertheless, a substantial number of inquiries have been received concerning the status of such water under the provisions of the act and the status, in interstate commerce, of commercially prepared foods in which fluoridated water has been used.

(c) The Federal Security Agency will regard water supplies containing fluorine, within the limitations recommended by the Public Health Service, as not actionable under the Federal Food, Drug, and Cosmetic Act. Similarly, commercially prepared foods within the jurisdiction of the act, in which a fluoridated water supply has been used in the processing operation, will not be regarded as actionable under the Federal law because of the fluorine content of the water so used, unless the process involves a significant concentration of fluorine from the water. In the latter instance the facts with respect to the particular case will be controlling.

(Sec. 701, 52 Stat. 1055; 21 U. S. C. 371)

Dated: July 17, 1952.

[SEAL] JOHN L. THURSTON,
Acting Administrator.

[F. R. Doc. 52-8041; Filed, July 22, 1952; 8:50 a. m.]

TITLE 26—INTERNAL REVENUE

Chapter I—Bureau of Internal Revenue, Department of the Treasury

Subchapter C—Miscellaneous Excise Taxes

[T. D. 5920; Regs. 132]

PART 325—EXCISE AND SPECIAL TAX ON WAGERING

REGISTRY, RETURN AND PAYMENT OF TAX

Regulations 132 amended to require persons liable for special (occupational) wagering tax to file returns and pay tax before commencing taxable activity and to file supplemental returns advising of all agents or employees engaged to receive wagers or with respect to all persons for whom wagers are received.

On June 3, 1952, notice of proposed rule making regarding amendment of § 325.50 of Regulations 132 was published in the FEDERAL REGISTER (17 F. R. 4988). No objection to the rules proposed having been received, § 325.50 of Regulations 132 is amended to read as follows:

§ 325.50 *Registry, return, and payment of tax.* (a) No person shall engage in the business of accepting wagers subject to the 10 percent excise tax imposed by section 3285 of the Internal Revenue Code (see § 325.24) until he has filed a return on Form 11-C and paid the special tax imposed by section 3290. Likewise, no person shall engage in receiving wagers for or on behalf of any person engaged in such business until he has filed a return on Form 11-C and paid the special tax imposed by section 3290 of the Internal Revenue Code. Filing of successive applications and payment of tax by such persons are required on or before July 1 of each year thereafter during which taxable activity continues. The return, with remittance, shall be filed with the collector of internal revenue for the district in which is located the taxpayer's office or principal place of business. If such taxpayer resides in the United States, but has no office or principal place of business in the United States, the return shall be filed with the collector of internal revenue for the district in which he resides. If the taxpayer has no office, residence, or principal place of business in the United States, the return shall be filed with the Collector of Internal Revenue, Baltimore, Maryland. The collector, upon request, will furnish the taxpayer proper forms which shall be filled out and signed as indicated therein.

(b) Each return shall show the taxpayer's full name. A person doing business under an alias, style, or trade name shall give his true name, followed by his alias, style, or trade name. In the case of a partnership, association, firm,

B 23

Title 21—Food and Drugs

CHAPTER I—FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

[Recodification Docket No. 9]

SUBCHAPTER C—DRUGS: GENERAL

Reorganization and Republication

The Commissioner of Food and Drugs, for the purposes of establishing an orderly development of informative regulations for the Food and Drug Administration, furnishing ample room for expansion of such regulations in years ahead, and providing the public and affected industries with regulations that are easy to find, read, and understand, has initiated a recodification program for Chapter I of Title 21 of the Code of Federal Regulations.

This is the ninth document in a series of recodification documents that will eventually include all regulations administered by the Food and Drug Administration.

This recodification document represents a reorganization of material remaining in Subchapter C—Drugs that has general applicability, rather than strictly human or animal use. In addition certain related sections under Parts 1 and 3 have been redesignated as part of the revised Subchapter C—Drugs: General.

The following table shows the relationship of the CFR section numbers under the former Subchapters A and C to their redesignation reflected in the new Parts 200 through 299:

Old Section	New Section	Old Section	New Section
1.100	299.5	3.21	250.102
1.101	201.6	3.22	200.101
1.101a	201.60	3.27	250.203
1.102	201.50	3.28	200.50
1.102a	201.61	3.29	201.307
1.102b	201.1	3.30	201.308
1.102c	201.51	3.35	201.303
1.102d	201.62	3.36	250.103
1.103	201.15	3.37	201.309
1.104	201.10	3.40	250.201
1.105	202.1	3.43	201.310
1.106(a)	201.5	3.44	201.311
1.106(b)	201.100	3.45	200.30
1.106(c)	201.105	3.48	250.106
1.106(d)	201.109	3.50	250.104
1.106(f)	201.110	3.52	250.107
1.106(g)	201.115	3.53	250.10
1.106(h)	201.116	3.56	201.405
1.106(i)	201.117	3.61	200.18
1.106(j)	201.119	3.62	299.4
1.106(k)	201.120	3.63	250.11
1.106(l)	201.122	3.64	250.12
1.106(m)	201.125	3.67	201.305
1.106(n)	201.127	3.71	250.100
1.106(o)	201.128	3.74	201.56
1.107	201.150	3.76	200.10
1.108(a) & (b)	201.16	3.77	299.35
1.108(c)	299.6	3.81	201.200
1.109	299.5	3.84	201.410
1.110	299.10	3.90	250.300
1.115	200.15	3.91	250.250
3.3	201.300	3.94	250.109
3.4	201.302	3.95	250.110
3.7	250.108	3.501	200.5
3.8	250.101	3.502	201.19
3.11	201.301	3.503	201.312
3.12	201.304	3.505	201.313
3.15	201.306	3.506	200.11
3.16	200.100	3.507	201.17
		3.508	201.18

Old Section	New Section	Old Section	New Section
3.509	201.314	133.11	211.58
3.510	201.315	133.12	211.110
3.512	200.31	133.13	211.60
3.513	200.7	133.14	211.62
3.514	201.55	133.15	211.115
3.515	201.160	133.100	225.1
3.516	250.105	133.101	225.20
3.518	201.161	133.102	225.30
132.1	207.3	133.103	225.10
132.2	207.20	133.104	225.42
132.3	207.21	133.105	225.102
132.4	207.22	133.106	225.40
132.5	207.25	133.107	225.80
132.6	207.30	133.108	225.58
132.7	207.31	133.109	225.110
132.8	207.35	133.110	225.115
132.9	207.37	133.200	226.1
132.10	207.26	133.201	226.20
132.11	207.39	133.202	226.30
132.31	207.40	133.203	226.10
132.51	207.65	133.204	226.42
133.1	210.3	133.205	226.102
133.2	211.1	133.206	226.40
133.3	211.20	133.207	226.80
133.4	211.30	133.208	226.58
133.5	211.10	133.209	226.110
133.6	211.42	133.210	226.115
133.7	211.101	133.300	229.25
133.8	211.40	138.1	299.3
133.9	211.55	138.2	299.20
133.10	211.80		

The changes being made are nonsubstantive in nature and for this reason notice and public procedure are not prerequisites to this promulgation. For the convenience of the user, the entire text of Parts 200, 201, 202, 207, 210, 211, 225, 226, 229, 250, 290, and 299 of Subchapter C is set forth below.

Dated: March 21, 1975.

SAM D. FINE,
Associate Commissioner for Compliance.

Therefore, 21 CFR is amended by redesignating portions of Parts 1 and 3 of Subchapter A and Parts 132, 133, and 138 of Subchapter C as Parts 200, 201, 202, 207, 210, 211, 225, 226, 229, 250, 290, and 299 of Subchapter C—Drugs: General, and republished to read as follows:

SUBCHAPTER C—DRUGS: GENERAL

Part

200—General

201—Labeling

202—Prescription Drug Advertising

207—Registration of Producers of Drugs and Listing of Drugs in Commercial Distribution

210—Current Good Manufacturing Practices in Manufacturing, Processing, Packing, or Holding of Drugs: General

211—Current Good Manufacturing Practice for Finished Pharmaceuticals

225—Current Good Manufacturing Practice for Medicated Feeds

226—Current Good Manufacturing Practice for Medicated Premixes

229—Current Good Manufacturing Practice for Certain Other Drug Products

250—Special Requirements for Specific Human Drugs

290—Controlled Drugs

299—Drugs; Official Names and Established Names

PART 200—GENERAL

Subpart A—General Provisions

Sec.

Subpart B—Manufacturing Procedures Affecting New Drug Status

Subpart C—Requirements for Specific Classes of Drugs

Subpart D—Suitability of Specific Drug Components

AUTHORITY: Sec. 701, 52 Stat. 1055; 21 U.S.C. 371, unless otherwise noted.

Subpart A—General Provisions

§ 200.5 Mailing of important information about drugs.

Manufacturers and distributors of drugs and the Food and Drug Administration occasionally are required to mail important information about drugs to physicians and others responsible for patient care. In the public interest, such mail should be distinctive in appearance so that it will be promptly recognized and read. The Food and Drug Administration will make such mailings in accordance with the specifications set forth in this section. Manufacturers and distributors of drugs are asked to make such mailings as prescribed by this section and not to use the distinctive envelopes for ordinary mail.

(a) Use first class mail and No. 10 white envelopes.

(b) The name and address of the agency or the drug manufacturer or distributor is to appear in the upper left corner of the envelope.

(c) The following statements are to appear in the far left third of the envelope front, in the type and size indicated, centered in a rectangular space approximately 3 inches wide and 2¼ inches high with an approximately ⅛-inch-wide border in the color indicated:

(1) When the information concerns a significant hazard to health, the statement:

IMPORTANT
DRUG
WARNING

The statement shall be in three lines, all capitals, and centered. "Important" shall be in 36 point Gothic Bold type. "Drug" and "Warning" shall be in 36 point Gothic Condensed type. The rectangle's

B24

code of federal regulations

Food and Drugs

21

PARTS 200 TO 299

Revised as of April 1, 1995

CONTAINING
A CODIFICATION OF DOCUMENTS
OF GENERAL APPLICABILITY
AND FUTURE EFFECT

AS OF APRIL 1, 1995

With Ancillaries

Published by
the Office of the Federal Register
National Archives and Records
Administration

as a Special Edition of
the Federal Register

B25

ing of section 503(b) of the Federal Food, Drug, and Cosmetic Act unless it is labeled with the legend "Caution—Federal law prohibits dispensing without prescription."

(e) Any drug for oral ingestion intended, represented, or advertised for the prevention or treatment of pernicious anemia or which purports to contain any substance or mixture of substances described in paragraph (d) of this section (other than diagnostic drugs containing radioactive cyanocobalamin) will be regarded as misbranded under sections 502(f)(2) and (j) of the act unless its labeling bears a statement to the effect that some patients afflicted with pernicious anemia may not respond to the orally ingested product and that there is no known way to predict which patients will respond or which patients may cease to respond to the orally ingested products. The labeling shall also bear a statement that periodic examinations and laboratory studies of pernicious anemia patients are essential and recommended.

(f) Under section 409 of the Federal Food, Drug, and Cosmetic Act, intrinsic factor and intrinsic factor concentrate are regarded as food additives. No food additive regulation nor existing extension of the effective date of section 409 of the act authorizes these additives in foods, including foods for special dietary uses. Any food containing added intrinsic factor or intrinsic factor concentrate will be regarded as adulterated within the meaning of section 402(a)(2)(C) of the act.

(g) Regulatory action may be initiated with respect to any article shipped within the jurisdiction of the act contrary to the provisions of this policy statement after the 180th day following publication of this statement in the FEDERAL REGISTER.

§250.203 Status of fluoridated water and foods prepared with fluoridated water.

(a) The program for fluoridation of public water supplies recommended by the Department of Health and Human Services, through the Public Health Service (Centers for Disease Control), contemplates the controlled addition of fluorine at a level optimum for the prevention of dental caries.

(b) Public water supplies do not ordinarily come under the provisions of the Federal Food, Drug, and Cosmetic Act. Nevertheless, a substantial number of inquiries have been received concerning the status of such water under the provisions of the act and the status, in interstate commerce, of commercially prepared foods in which fluoridated water has been used.

(c) The Department of Health and Human Services will regard water supplies containing fluorine, within the limitations recommended by the Environmental Protection Agency, as not actionable under the Federal Food, Drug, and Cosmetic Act. Similarly, commercially prepared foods within the jurisdiction of the act, in which a fluoridated water supply has been used in the processing operation, will not be regarded as actionable under the Federal law because of the fluorine content of the water so used, unless the process involves a significant concentration of fluorine from the water. In the latter instance the facts with respect to the particular case will be controlling.

[40 FR 14033, Mar. 27, 1975, as amended at 48 FR 11426, Mar. 18, 1983]

Subpart D—Requirements for Drugs and Cosmetics

§250.250 Hexachlorophene, as a component of drug and cosmetic products.

(a) *Antibacterial component.* The use of hexachlorophene as an antibacterial component in drug and cosmetic products has expanded widely in recent years. It is used in such products because of its bacteriostatic action against gram-positive organisms, especially against strains of staphylococcus; however, hexachlorophene offers no protection against gram-negative infections. In addition, the antibacterial activity depends largely on repeated use. A notice published in the FEDERAL REGISTER of April 4, 1972 (37 FR 6775), invited data on OTC antimicrobial ingredients, including hexachlorophene, for review by an OTC Drug Advisory Review Panel to be convened under the procedures set forth in the FEDERAL REGISTER of May 11, 1972

B 26

List of substances	Limitations
Monochlorobenzene Monochlorobenzene.	Not to exceed 500 parts per million as residual solvent in finished basic resin in paragraph (a)(1) of this section.
N-methyl-2-pyrrolidone.	Not to exceed 0.01 percent (100 parts per million) as residual solvent in finished basic resin in paragraph (a)(2) of this section.

* * * * *

Dated: May 17, 1996.

Fred R. Shank,

Director, Center for Food Safety and Applied Nutrition.

[FR Doc. 96-14697 Filed 6-10-96; 8:45 am]

BILLING CODE 4160-01-F

Food and Drug Administration

21 CFR Parts 200, 250, and 310

[Docket No. 95N-0310]

Revocation of Obsolete Regulations

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is revoking certain regulations that are obsolete or are no longer necessary to achieve public health goals. These regulations were among those identified for revocation in a page-by-page review conducted in response to the Administration's "Reinventing Government" initiative, which seeks to streamline government to ease the burden on regulated industry and consumers.

EFFECTIVE DATE: July 11, 1996.

FOR FURTHER INFORMATION CONTACT: Christine F. Rogers, Center for Drug Evaluation and Research (HFD-7), Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301-594-2041.

SUPPLEMENTARY INFORMATION:

I. Background

In the Federal Register of October 13, 1995 (60 FR 53480), FDA published a proposed rule to revoke certain regulations. This was done in response to the President's order to all Federal agencies to conduct a page-by-page review of all their regulations and to "eliminate or revise those that are outdated or otherwise in need of reform." The proposed rule contained a section-by-section analysis of all the regulations (21 CFR parts 100, 101, et al.) that FDA intended to revoke. This final rule pertains only to those regulations (21 CFR parts 200, 250, and 310) pertaining exclusively to the Center for Drug Evaluation and Research. No comments were received in response to the proposal to revoke these regulations.

II. Analysis of Impacts

FDA has examined the impacts of the final rule under Executive Order 12866 and the Regulatory Flexibility Act (Pub. L. 96-354). Executive Order 12866 directs agencies to assess all costs and benefits of available regulatory alternatives and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety, and other advantages; distributive impacts; and equity). The agency believes that this final rule, which is the revocation of certain regulations that are obsolete or are no longer necessary, is consistent with the regulatory philosophy and principles identified in the Executive Order. In addition, the final rule is not a significant regulatory action as defined by the Executive Order and so is not subject to review under the Executive Order.

The Regulatory Flexibility Act requires agencies to analyze regulatory options that would minimize any significant impact of a rule on small entities. Because this final rule is the revocation of certain regulations that are obsolete or are no longer necessary, the agency is not aware of any adverse impact this final rule will have on any small entities, and the agency certifies that the final rule will not have a significant economic impact on a substantial number of small entities. Therefore, under the Regulatory Flexibility Act, no further analysis is required.

III. Environmental Impact

The agency has determined under 21 CFR 25.24(a)(9) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

List of Subjects

21 CFR Part 200

Drugs, Prescription drugs.

21 CFR Part 250

Drugs.

21 CFR Part 310

Administrative practice and procedure, Drugs, Labeling, Medical devices, Reporting and recordkeeping requirements.

Therefore, under the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 *et seq.*) and under authority delegated to the Commissioner of Food and Drugs, 21 CFR parts 200, 250, and 310 are amended as follows:

PART 200—GENERAL

1. The authority citation for 21 CFR part 200 continues to read as follows:

Authority: Secs. 201, 301, 501, 502, 503, 505, 506, 507, 508, 515, 701, 704, 705 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 358, 360e, 371, 374, 375).

2. Sections 200.100 and 200.101 are removed and the heading for subpart D is reserved.

PART 250—SPECIAL REQUIREMENTS FOR SPECIFIC HUMAN DRUGS

3. The authority citation for 21 CFR part 250 continues to read as follows:

Authority: Secs. 201, 306, 402, 502, 503, 505, 601(a), 602(a) and (c), 701, 705(b) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 336, 342, 352, 353, 355, 361(a), 362(a) and (c), 371, 375(b)).

§ 250.104 [Removed]

4. Section 250.104 *Status of salt substitutes under the Federal Food, Drug, and Cosmetic Act* is removed.

§ 250.203 [Removed]

5. Section 250.203 *Status of fluoridated water and foods prepared with fluoridated water* is removed.

PART 310—NEW DRUGS

6. The authority citation for 21 CFR part 310 continues to read as follows:

Authority: Secs. 201, 301, 501, 502, 503, 505, 506, 507, 512-516, 520, 601(a), 701, 704, 705, 721 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e); secs. 215, 301, 302(a), 351, 354-360F of the Public Health Service Act (42 U.S.C. 216, 241, 242(a), 262, 263b-263n).

§ 310.101 [Removed]

7. Section 310.101 *FD&C Red No. 4; procedure for discontinuing use in new drugs for ingestion; statement of policy* is removed.

B27

ENVIRONMENTAL PROTECTION AGENCY

[OW-FRL-3410-1]

Drinking Water Technical Assistance; Termination of the Federal Drinking Water Additives Program

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: The Environmental Protection Agency (EPA), Office of Drinking Water (ODW), has operated an advisory program that gives technical assistance to concerned parties on the use of drinking water additives. On May 17, 1984, EPA proposed to terminate major elements of this Federal program and to assist in the establishment of a private-sector program which would offer assistance in evaluating drinking water additives. 49 FR 21004. EPA solicited proposals from qualified nongovernmental, nonprofit organizations for assistance under a cooperative agreement to establish a credible and efficient program in the private sector.

On September 17, 1985, EPA selected a consortium consisting of the National Sanitation Foundation (NSF), the American Water Works Association Research Foundation (AWWARF), the Conference of State Health and Environmental Managers (COSHEM), and the Association of State Drinking Water Administrators (ASDWA) to receive funds under a cooperative agreement to develop the private-sector program. EPA believes that the NSF-led program has proceeded satisfactorily. NSF Standard 60, covering many direct additives, was adopted on December 7, 1987; and NSF Standard 61, covering indirect additives, was adopted on June 3, 1988. Other standards are forthcoming. The NSF-led program has begun offering testing, certification, and listing services, as described in 49 FR 21004, for certain classes of products covered by these standards. Accordingly, as the NSF-led program becomes operational, EPA will phase out its activities in this area, as described in this notice.

DATE: Any written comments on implementing this notice should be submitted to the address below by September 6, 1988.

ADDRESSES: Submit comments to: Mr. Arthur H. Perler, Chief, Science and Technology Branch, Office of Drinking Water (WH-550D), U.S. Environmental Protection Agency, 401 M Street, SW., Washington, DC 20460. A copy of all comments will be available for review during normal business hours at the U.S. Environmental Protection Agency, Criteria and Standards Division, Science and Technology Branch, Room 931ET, 401 M Street, SW., Washington, DC 20460. For further information on the NSF-led private-sector program, including standards development and testing, certification, and listing services, contact: Director, Drinking Water Additives Program, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106; or call (313) 769-8010. For information on alternative testing, certification, and listing programs, contact individual State regulatory authorities or the American Water Works Association, Technical and Professional Department, 6666 Quincy Avenue, Denver CO, 80235, or call (303) 794-7711. For information on the directory of products certified as meeting the criteria in a NSF standard, contact the American Water Works Association Research Foundation, 6666 Quincy Avenue, Denver CO, 80235, or call (303) 794-7711.

FOR FURTHER INFORMATION CONTACT: Mr. Arthur H. Perler, Chief, Science and Technology Branch, Office of Drinking Water (WH-550D), U.S. Environmental Protection Agency, 401 M Street, SW., Washington, DC 20460, or call (202) 382-2022.

I. Introduction

The Safe Drinking Water Act (SDWA) (42 U.S.C. 300f *et seq.*) provides for enhancement of the safety of public drinking water supplies through the establishment and enforcement of national drinking water regulations. The Environmental Protection Agency (EPA) has the primary responsibility for establishing the regulations, and the States have the primary responsibility for enforcing such regulations. The regulations control contaminants in drinking water which may have any adverse effect on public health. Section 1412, 42 U.S.C. 300g-1. The regulations include maximum contaminant levels (MCLs) or treatment techniques and monitoring requirements for these contaminants. Sections 1401 and 1412; 42 U.S.C. 300f and 300g-1. EPA also promulgates monitoring requirements for unregulated contaminants. Section 1445; 42 U.S.C. 300j-4. In addition, EPA has broad authorities to provide technical assistance and financial assistance (e.g., grants, cooperative agreements) to States and to conduct research. Sections 1442, 1443, 1444; 42 U.S.C. 300j-1, 300j-2, 300j-3.

The Agency has established MCLs for a number of harmful contaminants that occur naturally or pollute public drinking water supplies. In addition to such contaminants, there is a possibility that drinking water supplies may be contaminated by compounds "added" to drinking water, either directly or indirectly, in the course of treatment and transport of drinking water. Public water systems use a broad range of chemical products to treat water supplies and to maintain storage and distribution systems. For instance, systems may directly add chemicals such as chlorine, alum, lime, and coagulant aids in the process of treating water to make it suitable for public consumption. These are known as "direct additives." In addition, as a necessary function of maintaining a public water system, storage and distribution systems (including pipes, tanks, and other equipment) may be fabricated from or painted, coated, or treated with products which may leach into or otherwise enter the water. These products are known as "indirect additives." Except to the extent that direct or indirect additives consist of ingredients or contain contaminants for which EPA has promulgated MCLs, EPA does not currently regulate the levels of additives in drinking water.

In 1979, EPA executed a Memorandum of Understanding (MOU) with the U.S. Food and Drug Administration (FDA) to establish and clarify areas of authorities with respect to control of additives in drinking water. 44 FR 42775, July 20, 1979. FDA is authorized to regulate "food additives" pursuant to the Federal Food, Drug, and Cosmetic Act (FFDCA). (21 U.S.C. 301 *et seq.*). Both agencies acknowledged in the MOU that "passage of the SDWA in 1974 repealed FDA's authority under the FFDCA over water used for drinking water purposes." The MOU stated that FDA would continue to have authority for taking regulatory action under the FFDCA to control additives in bottled drinking water and in water used in food and for food processing. The MOU went on to say that EPA had authority to control additives in public drinking water supplies.

While the SDWA does not require EPA to control the use of specific additives in drinking water, EPA has provided technical assistance to States and public water systems on the use of additives through the issuance of advisory opinions on the acceptability of many additive products. EPA has provided this technical assistance pursuant to its discretionary authority in section 1442(b)(1) to "collect and make available information pertaining to research, investigations and demonstrations with respect to

B 28

providing a dependable safe supply of drinking water together with appropriate recommendations in connection therewith." EPA has additional authorities under the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601 *et seq.*) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) that could be used to control additives in drinking water. TSCA authorizes EPA to regulate a new chemical substance before it is manufactured or any existing chemical substance before it is manufactured or processed for a use that EPA has determined to be a "significant new use." Although an additive product might come within the jurisdiction of TSCA, EPA has never invoked this authority. EPA has used its authority under FIFRA to control the use of pesticides, disinfectants, and certain other additives. For a more complete discussion of these authorities, see the MOU. 44 FR 42776.

In 1980, EPA declared a moratorium on the issuance of new advisory opinions on additives pending a review of past advisory opinions and the establishment of uniform test protocols and decision criteria. However, between 1980 and 1984, EPA continued to issue advisory opinions in cases where the new additive products were virtually identical to products previously reviewed. Resource constraints and the need to implement mandatory provisions of the SDWA precluded the Agency from implementing the comprehensive program originally envisioned for the issuance of additives advisory opinions. Thus, the Agency was not able to review the technical data supporting previous submissions (approximately 2,300 products from 525 manufacturers) nor was it able to develop test protocols or decision criteria for the consistent evaluation of new products. The result has been long delays in processing manufacturer petitions, inability to review and accept completely new products, and acceptance of products simply because they were virtually identical to older products. Hence, few products have been thoroughly evaluated for the safety of their formulations based on the latest scientific information.

Recognizing the need for continuing technical assistance in evaluating additive products and for providing advice to States and public water systems on the toxicological aspects of additive products, the Agency proposed to terminate its attempts to institute a formal advisory program, and to solicit proposals from nongovernmental, nonprofit organizations to establish such a program in the private sector. The Agency believed that the proposal to assist in the establishment of a private-sector program was consistent with, and would best serve the goals of, the SDWA.

On May 17, 1984, EPA formally announced its intention to transfer the program to the private sector, which would function as to many other voluntary product-standard programs. 49 FR 21004. This was accomplished by requesting proposals from qualified organizations or consortia of organizations for the competitive award of a cooperative agreement designed to provide incentive for the establishment of a private-sector program. The 1984 notice stated that:

- EPA expected the activity to be self-supporting.
- EPA would maintain an active interest in the development of the program, without assuming responsibility for or directing its approach.
- EPA would continue to establish regulations under the SDWA, FIFRA, and/or TSCA, as needed, for chemicals in treated, distributed drinking water that may originate as additives.
- Establishment of such a program would be consistent with the Administration's initiatives in the area of regulatory reform and offered an opportunity for an innovative alternative to regulation.

The May 1984 notice requested public comments on the proposal and solicited applications from qualified nongovernmental, nonprofit organizations for partial funding of the developmental phase of the program under a cooperative agreement. The response to the solicitation for comments indicated strong public support for the proposed approach. EPA received 106 public comments on the proposal. All but six supported this "third-party" approach. However, despite the Agency's open competition, EPA received only one application for financial assistance. The applicant was a consortium, led by the National Sanitation Foundation, which included the American Water Works Association Research Foundation, the Conference of State Health and Environmental Managers, and the Association of State Drinking Water Administrators. This single proposal met all of the basic criteria articulated in the May 1984 notice. Furthermore, EPA believed that the single applicant was very likely to succeed, because it represented an organization experienced in private-sector consensus standard-setting, State regulators, and water utilities.

EPA awarded the cooperative agreement to the NSF consortium on September 17, 1985, and committed funding of $185,000 to NSF over a three-year period. The non-Federal (consortium and participating industry) contribution during the first three years of the program was projected to be approximately $1.4 million.

The NSF program has the following major objectives:

- To develop systematic, consistent, and comprehensive voluntary consensus standards for public health safety evaluation of all products (previously EPA-accepted as well as new) intended for use in drinking water systems.
- To obtain broad-based participation in the standard-setting program from industry, States, and utilities.
- To provide for regular periodic review, update, and revision of the standards.
- To undertake needed research, testing, evaluation, and inspections and to provide the followup necessary to maintain the program.
- To establish a separate program for testing, evaluation, certification, and listing of additive products.
- To widely disseminate information about the program, and to make information about conforming products available to users.
- To maintain the confidentiality of all proprietary information.
- To fully establish the third-party program on a self-supporting basis.

NSF's established standard-setting process utilizes a tiered structure. Each standard is drafted by a task group and then presented to a Joint Committee, which includes 12 industry, 12 user, and 12 regulatory members. Following successful Joint Committee balloting, standards are reviewed by the Council of Public Health Consultants, which is a high level advisory group consisting of technical and policy experts from regulatory agencies and academia.

NSF has established task groups to develop standards for the product categories listed below. Each task group includes a member representing the regulatory agencies and a member representing the utilities. All manufacturers expressing interest in a particular product task group may participate as members of that group. Therefore, task group membership is predominately manufacturers. In addition, a group of health effects consultants is addressing the toxicological and risk considerations for various product categories. NSF's role in the standard-setting process is administrative, that is, to bring together experts from government, industry,

B29

utilities, users, and other relevant groups so that a standard which reflects a consensus of these interests can be developed. In addition, NSF staff provide technical leadership and laboratory support. Product categories and correspoding task groups are:

- Protective Materials.
- Chemicals for Corrosion and Scale Control, Softening, Precipitation, Sequestering, and pH Adjustment.
- Coagulation and Flocculation Chemicals.
- Miscellaneous Treatment Chemicals.
- Joining and Sealing materials.
- Process Media.
- Pipes and Related Products.
- Disinfection and Oxidation Chemicals.
- Mechanical Devices.

All of the task groups have made satisfactory progress during the term of the cooperative agreement. In addition, the health effects consultants have endorsed the bases of the standards. Standards have been drafted for all product categories, and final standards were published and implemented as follows:

Standard 60, December 1987

- Chemicals for Corrosion and Scale Control, Softening, Precipitation, Sequestering, and pH Adjustment.
- Disinfection and Oxidation Chemicals.
- Miscellaneous Treatment Chemicals (selected).

Standard 61, June 1988

- Process Media.

Development of the remaining standards is on schedule, and publication and implementation are expected on the following schedule:

Standards 60 and 61, expected October 1988

- Protective Materials.
- Coagulation and Flocculation Chemicals.
- Miscellaneous Treatment Chemicals (additional).
- Joining and Sealing Materials.
- Pipes and Related Products.
- Mechanical Devices.

EPA believes that the NSF program is successfully pursuing all of its objectives. Furthermore, the program is strongly supported by user and regulatory sectors. AWWARF, COSHEM, ASDWA, the Great Lakes Upper Mississippi River Board, the American Water Works Association (AWWA) (including the Utilities and Standards Councils and the Regulatory Agencies Division), and the Association of Metropolitan Water Agencies, among others, have voiced strong support for the third-party program. The AWWA recently joined the NSF-led consortium and urged EPA to support national uniform accreditation of certifying entities for additives products. To date, more than 60 manufacturers are full participants in the standard-setting program.

The cooperative agreement between EPA and the consortium requires NSF to establish both a standard-setting program and a service for testing, certification, and listing. These are completely separate activities. EPA's intent is to support the development of a widely accepted uniform standard for each category of products while encouraging the development of competing sources for testing, certification, and listing. The cooperative agreement assures that at least one sound and reliable product-evaluation service will be available to manufacturers, i.e., the consortium. However, the consortium's standards will allow for entities other than NSF to be evaluators of products.

EPA recognizes the authority and responsibility of the individual States to determine the acceptability of drinking water additives. Hence, it is up to the States and utilities to determine the suitability of any "third-party" certification. AWWARF will maintain a directory of products approved by all organizations claiming to conduct evaluations under Standards 60 and 61. However, AWWARF will not judge the competence or reliability of these organizations.

II. Announcement of Phase-Down of EPA's Additives Program

During the developmental phase of the NSF consortium's program, EPA has continued to review products and process requests for advisory opinions on a limited basis. The May 1984 notice stated that, "EPA does not intend to develop further interim administrative procedures, testing protocols or decision criteria for future evaluation of additive products. The use of existing informal criteria will continue until a third-party or alternative program is operational * * *. EPA may not be able to process all requests for opinions on additive products before the establishment of a cooperative agreement with a third party. The large volume of currently pending requests makes it unlikely that additional requests will be completely processed by that date." Likewise, EPA, in its acknowledgment letters to manufacturers requesting opinions on new products, explains that the Agency is, "* * * making a concerted effort to process petitions as quickly as possible. However, EPA may not be able to process your request for an opinion on an additive product before the establishment of an alternative program as described in the Federal Register, Vol. 49, No. 97, 21003–8, May 17, 1984." Product reviews and issuance of advisory opinions have been limited to:

- Products composed entirely of other products which EPA had previously determined to be acceptable;
- Products composed entirely of ingredients which have been determined to be acceptable by EPA or the FDA, or other Federal agencies, for addition to potable water or aqueous foods;
- Products composed entirely of ingredients listed in the "Water Chemicals Codex," National Academy of Sciences, November 1982, and in the "Water Chemicals Codex: Supplementary Recommendations for Direct Additives," National Academy of Sciences, 1984;
- Certain other products of particular interest to EPA or to other Federal agencies; and
- Products which, if effectively excluded from the marketplace by lack of approval, might jeopardize public health or safety.

Continued processing of petitions during the development of the private-sector program minimized disruption of the marketplace from the viewpoint of manufacturers whose business depended in part on EPA acceptance of products, users who required water treatment products for the production of safe drinking water, and State officials who rely on the advice of EPA.

EPA believes that NSF is moving expeditiously and on schedule toward the full establishment of a third-party program covering products intended for use in drinking water systems. Priorities for standards development and implementation of a testing, certification, and listing program for various product categories have been based upon need, interest, complexity, and availability of information for developing standards. Direct drinking water additives were assigned high priority for the following reasons: (1) Use of direct additives is widespread in drinking water systems, so there are large population exposures to these chemicals; (2) as direct additives to drinking water, they present greater potential for water contamination than indirect mechanisms (e.g., migration from protective paints in pipes and storage tanks); and (3) the National Academy of Sciences' *Water Chemicals Codex* provided a good starting point for development of standards.

B 30

As originally planned, EPA is beginning to phase out the Agency's additives evaluation program. Thus, EPA will not accept new petitions or requests for advisory options after the date of this notice. While EPA will continue to process requests which are pending and those received on or before July 7, 1988, petition evaluations not completed by October 4, 1988, will be returned to the submitter. After that date, EPA will no longer evaluate additive products.

Petitions which are completely evaluated by October 5, 1988, will be added to the quarterly list of acceptable products published shortly after that date. That quarterly list will be the last such list issued by EPA. On April 7, 1990, EPA will withdraw its list of acceptable products, and the list and the advisories on these additives will expire. This means that: (1) The various lists published by EPA under the titles *Report on Acceptable Drinking Water Additives, Report on Coagulant Aids for Water Treatment, Report on Concrete Coatings/Admixture for Water Treatment, Report on Detergents, Sanitizers and Joint Lubricants for Water Treatment, Report on Evaporative Suppressants for Water Treatment, Report on Liners/Grouts/Hoses and Tubings for Water Treatment, Report on Miscellaneous Chemicals for Water Treatment, Report on Protectivce Paints/Coatings for Water Treatment,* and any and all other lists of drinking water products issued by EPA or its predecessor agencies regarding drinking water additives will be invalid after April 7, 1990; and (2) advisory opinions on drinking water additives issued by EPA and predecessor agencies will be invalid after that date.

EPA believes that, while in the past every effort has been made to provide the best possible evaluations, all products should be evaluated against carefully developed and considered nationally uniform standards. Many of the currently listed products were evaluated and accepted up to 20 years ago and have not been reevaluated since that time. Numerous products have been accepted because they were virtually identical to or were repackagings of older products. The result is that few products have been completely evaluated for the safety of their original or current formulations vis-a-vis the latest toxicological, chemical, and engineering information. A uniform evaluation of all products, old and new, will result in consistent quality of products, and will assure fair and equitable treatment to all manufacturers and distributors.

Henceforth, parties desiring to have existing or new products evaluated against the NSF standards should contact NSF or other organizations offering such evaluations. To contact NSF about the drinking water additives program write to: David Gregorka, National Sanitation Foundation, P.O. Box 1468, Ann Arbor, MI 48106, or call (313) 769-8010. Information on alternatives to NSF evaluation may be obtained by contacting State regulatory agencies or the AWWA, Technical and Professional Department, 6666 Quincy Avenue, Denver Co, 80235, or call (303) 794-7711, which is addressing certifier accreditation.

EPA believes that the 21 months between today and the expiration date of EPA's last list is sufficient time for manufacturers to submit their products to NSF or other certification entities for evaluation. The first NSF list will be published prior to April 7, 1990, thereby preventing any disruption in the marketplace. Furthermore, NSF had indicated that it will consider current EPA and other regulatory evaluations when evaluating products in order to ensure a smooth transition. States may choose to rely on the last EPA quarterly list of products until their individual programs for accepting private-sector certification are fully implemented.

Parties desiring to market drinking water additive products are reminded that the individual States have the authority to regulate the sale and/or use of specific products as they see fit. Thus, reliance upon a particular standard or organization to certify that a product complies with a particular standard must be acceptable to the State in which the supplier wishes to do business.

Discontinuation of the additives program at EPA does not relieve the Agency of its statutory responsibilities. If contamination resulting from third-party sanctioned products occurs or seems likely, EPA will address that issue with appropriate drinking water regulations or other actions authorized under the SDWA. EPA is a permanent member of the NSF program Steering Committee, and senior EPA staff and management will continue to participate in this and other programs designed to assure that high-quality products are employed in the treatment of public drinking water. Also, the Agency will continue to sponsor research on contaminants introduced in public water supplies during water treatment, storage, and distribution.

III. Comments

Although this notice does not include a proposed or final regulation, EPA welcomes comments and suggestions that would assist the Agency in implementing the additives program phasedown. Please address all comments and suggestions to: Mr. Arthur H. Perler, Chief, Science and Technology Branch, Office of Drinking Water (WH-550D), U.S. Environmental Protection Agency, 401 M Street, SW., Washington, DC 20460.

Date: June 16, 1988.

William Whittington,

Acting Assistant Administrator for Water.

[FR Doc. 88-15232 Filed 7-6-88; 8:45 am]

BILLING CODE 6560-50-M

B31

Federal facilities. Prior to making a final recommendation to the Administrator, U.S. EPA, the Regional Administrator, Region V, is providing opportunity for public comment on the State of Wisconsin request. Any interested person may comment upon the State request by writing to the U.S. EPA, Region V Office, 230 South Dearborn Street, Chicago, Illinois 60604, Attention: Permit Branch. Such comments will be made available to the public for inspection and copying. All comments or objections received by August 22, 1979, will be considered by U.S. EPA before taking final action on the Wisconsin request for authority to issue permits to Federal facilities.

The State's request, related documents, and all comments received are on file and may be inspected and copied (@ 20 cents/page) at the U.S. EPA, Region V Office, in Chicago.

Copies of this notice are available upon request from the Enforcement Division of U.S. EPA, Region V, by contacting Dorothy A. Price, Public Notice Clerk (312–353–2105), at the above address.

Dated: July 13, 1979.

John McGuire,
Regional Administrator.

[FR Doc. 79-22872 Filed 7-19-79; 8:45 am]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

ENVIRONMENTAL PROTECTION AGENCY

[FRL 1275–4]

Drinking Water Technical Assistance; Implementation Plan for Control of Direct and Indirect Additives to Drinking Water and Memorandum of Understanding Between the Environmental Protection Agency and the Food and Drug Administration

AGENCY: Environmental Protection Agency and Food Drug Administration.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) have executed a memorandum of understanding (MOU) with regard to the control of direct and indirect additives to and substances in drinking water. The purpose of the MOU is to avoid the possibility of overlapping jurisdiction between EPA and FDA with respect to control of drinking water additives. The agreement became effective on June 22, 1979.

ADDRESS: Submit comments to: Victor J. Kimm, Deputy Assistant Administrator for Drinking Water, Environmental Protection Agency (WH–550), Washington, D.C. 20460.

FOR FURTHER INFORMATION CONTACT: David W. Schnare, Ph.D., Office of Drinking Water (WH–550), Environmental Protection Agency, Washington, D.C. 20460, (202) 755–5643; or Gary Dykstra, Enforcement Policy Staff (HFC–22), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, (301) 443–3470.

SUPPLEMENTARY INFORMATION: In the spirit of interagency cooperation and to avoid the possibility of overlapping jurisdiction over additives and other substances in drinking water, FDA and EPA have entered into a memorandum of understanding to avoid duplicatve and inconsistent regulation. In brief, the memorandum provides that EPA will have primary responsibility over direct and indirect additives and other substances in drinking water under the Safe Drinking Water Act, the Toxic Substances Control Act, and the Federal Insecticide, Fungicide and Rodenticide Act. FDA will have responsibility for water, and substances in water, used in food and for food processing and for bottled water under the Federal Food, Drug and Cosmetic Act.

Pursuant to the notice published in the Federal Register of October 3, 1974, (39 FR 35697) stating that future memoranda of understanding, and agreements between FDA and others would be published in the Federal Register, the following memorandum of understanding is issued:

Memorandum of Understanding Between the Environmental Protection Agency and the Food and Drug Administration

I. Purpose

This Memorandum of Understanding establishes an agreement between the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) with regard to the control of direct and indirect additives to and substances in drinking water.

EPA and FDA agree:

(1) That contamination of drinking water from the use and application of direct and indirect additives and other substances poses a potential public health problem;

(2) That the scope of the additives problem in terms of the health significance of these contaminants in drinking water is not fully known;

(3) That the possibility of overlapping jurisdiction between EPA and FDA with respect to control of drinking water additives has been the subject of Congressional as well as public concern;

(4) That the authority to control the use and application of direct and indirect additives to and substances in drinking water should be vested in a single regulatory agency to avoid duplicative and inconsistent regulation;

(5) That EPA has been mandated by Congress under the Safe Drinking Water Act (SDWA), as amended, to assure that the public is provided with safe drinking water;

(6) That EPA has been mandated by Congress under the Toxic Substances Control Act (TSCA) to protect against unreasonable risks to health and the environment from toxic substances by requiring, *inter alia*, testing and necessary restrictions on the use, manufacture, processing, distribution, and disposal of chemical substances and mixtures;

(7) That EPA has been mandated by Congress under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, to assure, *inter alia*, that when used properly, pesticides will perform their intended function without causing unreasonable adverse effects on the environment; and,

(8) That FDA has been mandated by Congress under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended, to protect the public from, *inter alia*, the adulteration of food by food additives and poisonous and deleterious substances.

It is the intent of the parties that;

(1) EPA will have responsibility for direct and indirect additives to and other substances in drinking water under the SDWA, TSCA, and FIFRA; and,

(2) FDA will have responsibility for water, and substances in water, used in food and for food processing and responsibility for bottled drinking water under the FFDCA.

II. Background

(A) *FDA Legal Authority.* "Food" means articles used for food or drink for man or other animals and components of such articles. (FFDCA § 201(f)). Under Section 402, *inter alia*, a food may not contain any added poisonous or deleterious substance that may render it injurious to health, or be prepared, packed or handled under unsanitary conditions. Tolerances may be set, under Section 403, limiting the quantity of any substance which is required for the production of food or cannot be avoided in food. FDA has the authority under Section 409 to issue food additive regulations approving, with or without conditions, or denying the use of a "food additive." That term is defined in Section 201(s) to include any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food, if such substance is not generally recognized as safe.

In the past, FDA has considered drinking water to be a food under Section 201(f). However, both parties have determined that the passage of the SDWA in 1974 implicitly repealed FDA's authority under the FFDCA over water used for drinking water purposes. Under the express provisions of Section 410

B32

of the FFDCA. FDA retains authority over bottled drinking water. Furthermore, all water used in food remains a food and subject to the provisions of the FFDCA. Water used for food processing is subject to applicable provisions of FFDCA. Moreover, all substances in water used in food are added substances subject to the provisions of the FFDCA, but no substances added to a public drinking water system before the water enters a food processing establishment will be considered a food additive.

(B). *EPA Legal Authority.* The SDWA grants EPA the authority to control contaminants in drinking water which may have any adverse effect on the public health, through the establishment of maximum contaminant levels (MCLs) or treatment techniques, under Section 1412, which are applicable to owners and operators of public water systems. The expressed intent of the Act was to give EPA exclusive control over the safety of public water supplies. Public water systems may also be required by regulation to conduct monitoring for unregulated contaminants under Section 1445 and to issue public notification of such levels under Section 1414(c).

EPA's direct authority to control additives to drinking water apart from the existence of maximum contaminant levels or treatment techniques is limited to its emergency powers under Section 1431. However, Section 1442(b) of the act authorizes EPA to "collect and make available information pertaining to research, investigations, and demonstrations with respect to providing a dependably safe supply of drinking water together with appropriate recommendations therewith."

TSCA gives EPA authority to regulate chemical substances, mixtures and under some circumstances, articles containing such substances or mixtures. Section 4 permits EPA to require testing of a chemical substance or mixture based on possible unreasonable risk of injury to health or the environment, or on significant or substantial human or environmental exposure while Section 8 enables EPA to require submission of data showing substantial risk of injury to health or the environment, existing health and safety studies, and other data. For new chemical substances, and significant new uses of existing chemical substances, Section 5 requires manufacturers to provide EPA with premanufacturing notice. Under Section 6 the manufacture, processing, distribution, use, and disposal of a chemical substance or mixture determined to be harmful may be restricted or banned. Although Section 3(2)(B) of TSCA excludes from the definition of "chemical substance" food and food additives as defined under FFDCA, the implicit repeal by the SDWA of FDA's authority over drinking water enables EPA to regulate direct and indirect additives to drinking water as chemical substances and mixtures under TSCA.

The FIFRA requires EPA to set restrictions on the use of pesticides to assure that when used properly, they will not cause unreasonable adverse effects on the environment. EPA may require, *inter alia*, labeling which specifies how, when, and where a pesticide may be legally used. In addition, EPA has, under Section 409 of the FFDCA, required FIFRA registrants at times to obtain a food additive tolerance before using a pesticide in or around a drinking water source. Such tolerances establish further restrictions on the use of a pesticide which are enforceable against the water supplier as well as the registrant of the pesticide.

III. Terms of Agreement

(A) EPA's responsibilities are as follows:

(1) To establish appropriate regulations, and to take appropriate measures, under the SDWA and/or TSCA, and FIFRA, to control direct additives to drinking water (which encompass any substances purposely added to the water), and indirect additives (which encompass any substances which might leach from paints, coatings or other materials as an incidental result of drinking water contact), and other substances.

(2) To establish appropriate regulations under the SDWA to limit the concentrations of pesticides in drinking water; the limitations on concentrations and types of pesticides in water are presently set by EPA through tolerances under Section 409 of the FFDCA.

(3) To continue to provide technical assistance in the form of informal advisory opinions on drinking water additives under Section 1442(b) of the SDWA.

(4) To conduct and require research and monitoring and the submission of data relative to the problem of direct and indirect additives in drinking water in order to accumulate data concerning the health risks posed by the presence of these contaminants in drinking water.

(B). FDA's responsibilities are as follows:

(1) To take appropriate regulatory action under the authority of the FFDCA to control bottled drinking water and water, and substances in water, used in food and for food processing;

(2) To provide assistance to EPA to facilitate the transition of responsibilities, including:

(a) To review existing FDA approvals in order to identify their applicability to additives in drinking water.

(b) To provide a mutually agreed upon level of assistance in conducting literature searches related to toxicological decision making.

(c) To provide a senior toxicologist to help EPA devise new procedures and protocols to be used in formulating advice on direct and indirect additives to drinking water.

IV. Duration of Agreement

This Memorandum of Understanding shall continue in effect unless modified by mutual consent of both parties or terminated by either party upon thirty (30) days advance written notice to the other.

This Memorandum of Understanding will become effective on the date of the last signature.

Dated: June 13, 1979.

Douglas M. Costle,

Administrator, Environmental Protection Agency.

Dated: June 22, 1979.

Donald Kennedy,

Administrator, Food and Drug Administration.

Implementation Plan

EPA is concerned that direct and indirect additives may be adding harmful trace chemical contaminants into our Nation's drinking water during treatment, storage and distribution. Direct additives include such chemicals as chlorine, lime, alum, and coagulant aides, which are added at the water treatment plant. Although these chemicals themselves may be harmless, they may contain small amounts of harmful chemicals if their quality is not controlled. Indirect additives include those contaminants which enter drinking water through leaching, from pipes, tanks and other equipment, and their associated paints and coatings. This notice is being published in the Federal Register to solicit public comment on EPA's implementation plan to assess and control direct and indirect additives in drinking water.

Legal Authorities

EPA and the Food and Drug Administration (FDA) signed a Memorandum of Understanding which recognizes that regulatory control over direct and indirect additives in drinking water is placed in EPA. The two agencies agreed that the Safe Drinking Water Act's passage in 1974 implicitly repealed FDA's jurisdiction over drinking water as a 'food' under the Federal Food, Drug and Cosmetic Act (FFDCA). Under the agreement, EPA now retains exclusive jurisdiction over drinking water served by public water supplies, including any additives in such water. FDA retains jurisdiction over bottled drinking water under Section 410 of the FFDCA and over water (and substances in water) used in food or food processing once it enters the food processing establishment.

In implementing its new responsibilities, EPA may utilize a variety of statutory authorities, as appropriate. The authorities are identified in Appendix A.

Under the Safe Drinking Water Act, EPA has authority to set and enforce maximum contaminant levels and treatment techniques in drinking water for ubiquitous contaminants, to conduct research, to offer technical assistance to States and to protect against imminent

B 33

hazards should such situations arise. Under the Toxic Substances Control Act, EPA has authority to review all new chemicals proposed for use related to drinking water, to mandate toxicological testing of existing and new chemicals where there is evidence that such materials may pose an unreasonable risk to health and the environment as well as authority to limit some or all uses of harmful chemicals. Pesticide use is regulated by EPA under the Federal Insecticide, Fungicide and Rodenticide Act. Thus, EPA believes it has adequate authority to deal with additives to drinking water where they may pose a problem.

Past Actions

For more than ten years, the Public Health Service and other organizations which have become part of EPA have provided advisory opinions on the toxicological safety of a variety of additives to drinking water. These historical informal opinions reflect a variety of information provided by manufacturers and reflect changing toxicological concerns over the years. As such, they will require detailed review over the next few years.

General Approach

EPA intends to begin its responsibility over additives to drinking water with a series of analytical studies to determine the composition and significance of the health risks posed by contaminants related to direct and indirect additives to drinking water. A first step in this process will be monitoring studies of the contaminants actually getting into drinking water from generic categories of additives like bulk chemicals, paints and coatings, pipes and equipment.

In the initial six to twelve months, EPA will develop interim administrative procedures, testing protocols, and decision criteria for future toxicological advisories to the States. These will be distributed for public comment once they are developed. All existing opinions will remain in effect until a general review of past opinions can be undertaken using the new procedures. During this development phase, no new opinions will be rendered unless a proposed product can be shown to be virtually identical to a product for which an opinion has already been rendered, on the basis of chemical formulation and production process. New products or new uses of existing products which are proposed for use in drinking water will be subject to the pre-manufacture notice procedures of TSCA.

A more detailed outline of the steps to be taken by EPA follows.

1. *Problem Definition.*—EPA will contract for *in situ* monitoring to determine use patterns and the contribution of trace contaminants to drinking water from:

a. bulk chemicals.

b. generic classes of paints and coatings.

c. pipes and equipment.

d. coagulant aids.

EPA has already contracted with the National Academy of Sciences to develop a CODEX system of quality control standards for chemicals (direct additives) used in the treatment of drinking water. This effort will take about three years to complete. When finished, the CODEX system, modeled on the existing FDA-inspired CODEX system for chemicals used in processing food, will be largely self-enforcing.

For the indirect additives listed in items b and c above, considerable effort will be expended to identify the trace contaminants involved before the related health risks can be fully evaluated and appropriate recommendations for future use can be assessed.

2. *Review of Past Advisories.*—The same data base derived from *in situ* monitoring will serve as a basis for a structured reassessment of past toxicological advisories which will be conducted by generic classes of use e.g., paints, coagulant aides, etc. Past opinions will be reviewed to insure conformance with and satisfaction of new test protocols and decision criteria that will be developed.

3. *Future Toxicological Advisories.*—Once initial procedures, test protocols and decision criteria are developed, EPA will resume offering toxicological opinions to the States.

General Policy

In assessing additives to drinking water, EPA will be guided by a policy of reducing public health risks to the degree it is feasible to do so. In such determinations, EPA will evaluate the risks and benefits associated with the materials of concern and their substitutes. Economic impacts of agency actions will also be analyzed.

Notwithstanding these procedures, EPA would use its authorities to protect against any direct or indirect additive to drinking water when data and information indicate that the use of any additive may pose an undue risk to public health.

Implementation

To fulfill this program, resources from the Office of Drinking Water, the Office of Research and Development, and the Office of Toxic Substances will be used. In addition, EPA looks forward to the cooperation of FDA and other Federal regulatory bodies. EPA intends to involve interested industry groups, independent testing groups, State regulatory bodies, interested members of the public, and industry standards groups, in a continued effort to ensure the safety of the Nation's drinking water.

Finally, EPA may recommend specialized legislative authority to regulate additives to drinking water should a situation arise for which legal authorities prove inadequate.

Lead responsibility for this new Federal initiative will be in EPA's Office of Drinking Water. Public comments on any or all aspects of the proposed program are requested, and should be directed to the address given in the opening sections of this notice.

Dated: July 13, 1979.

Thomas C. Jorling,

Assistant Administrator for Water and Waste Management.

Appendix A

Safe Drinking Water Act

Section 1412—establishment of national primary drinking water regulations applicable to public water systems to control contaminants in drinking water which may have any adverse effect on human health. This may include maximum contaminant levels, treatment techniques, monitoring requirements, and quality control and testing procedures.

Section 1431—use of emergency powers where a contaminant which is present in water, or is likely to enter a public water system, may present an imminent and substantial endangerment to the health of persons.

Section 1445—establishment of monitoring and reporting requirements applicable to public water systems.

Section 1450—authority to prescribe such regulations as are necessary or appropriate to carry out the Administrator's functions under the Act.

Toxic Substances Control Act

Section 4—testing of chemical substances and mixtures.

Section 5—pre-manufacture notice required for new chemicals or significant new uses.

Section 6—regulation of hazardous chemical substances and mixtures which pose an unreasonable risk of injury to health or the environment, including restrictions on manufacture, processing, distribution, and use.

B 34

Section 7—imminent hazards authority including seizure and other relief through civil court action.

Section 8—reporting and retention of information as required by the Administrator, including health and safety studies and notice to the Administrator of substantial risks.

Section 10—research and development. Development of systems for storing, retrieving and disseminating data.

Section 11—inspections and subpenas and other enforcement and general administration provisions therein.

Federal Insecticide, Fungicide and Rodenticide Act

Section 3—registration of pesticides, including imposition of restrictions and labeling requirements.

Section 6—suspension and cancellation procedures.

[FR Doc. 79-22222 Filed 7-18-79; 8:45 am]

BILLING CODE 6560-01-M

BILLING CODE 4110-03-M

FEDERAL COMMUNICATIONS COMMISSION

[Report No. A-1a]

FM Broadcasting Applications Accepted for Filing and Notification of Cut-off Date; Erratum

Released: July 12, 1979.

The FM Application listed below was inadvertently included on the acceptance/cut-off notice, Report No. A-1, BC Mimeo No. 18676, released on June 25, 1979.

BPH-790108AE (New): Creston, Pennsylvania, Sherlock-Hart Broadcasting, Inc.
Req.: 94.3 MHz, Channel 232A
ERP: 0.600 kW, HAAT: 600 feet.

Accordingly, the application is removed from the acceptance/cutoff list and the August 8, 1979, cutoff date is deleted.

Federal Communications Commission.

William J. Tricarico,

Secretary.

[FR Doc. 79-22422 Filed 7-19-79; 8:45 am]

BILLING CODE 6712-01-M

FEDERAL LABOR RELATIONS AUTHORITY

Official Time of Employees Involved in Negotiating Collective Bargaining Agreements

AGENCY: Federal Labor Relations Authority.

ACTION: Notice Relating to Official Time.

SUMMARY: This notice principally relates to the interpretation of section 7131 of the Federal Service Labor-Management Relations Statute (92 Stat. 1214) on the questions of whether employees who are on official time under this section while representing an exclusive representative in the negotiation of a collective bargaining agreement are entitled to payments from agencies for their travel and per diem expenses, and whether the official time provisions of section 7131(a) of the Statute encompass all negotiations between an exclusive representative and an agency, regardless of whether such negotiations pertain to the negotiation or renegotiation of a basic collective bargaining agreement. The notice further invites interested persons to address the impact, if any, of section 7135(a)(1) of the Statute (92 Stat. 1215) on such interpretation, and to submit written comments concerning these matters.

DATE: Written comments must be submitted by the close of business on August 24, 1979, to be considered.

ADDRESS: Send written comments to the Federal Labor Relations Authority, 1900 E Street, NW., Washington, D.C. 20424.

FOR FURTHER INFORMATION CONTACT: Harold D. Kessler, Deputy Executive Director, 1900 E Street, NW., Washington, D.C. 20424, (202) 632-3920.

SUPPLEMENTARY INFORMATION: The Federal Labor Relations Authority was established by Reorganization Plan No. 2 of 1978, effective January 1, 1979 (43 FR 36037). Since January 11, 1979, the Authority has conducted its operations under the Federal Service Labor-Management Relations Statute (92 Stat. 1191).

Upon receipt of requests and consideration thereof, the Authority has determined, in accordance with 5 CFR 2410.3(a) (1978) and sections 7105 and 7135(b) of the Statute (92 Stat. 1196, 1215), that an interpretation is warranted concerning section 7131 of the Statute (92 Stat. 1214). Interested persons are invited to express their views in writing on this matter, as more fully explained in the Authority's notice set forth below:

To Heads of Agencies, Presidents of Labor Organizations and Other Interested Persons

The Authority has received a request from the American Federation of Government Employees (AFGE) for a statement of policy and guidance concerning whether employees representing an exclusive representative in the negotiation of a collective bargaining agreement are entitled to payments from agencies for their travel and per diem expenses under the official time provisions of section 7131 of the Federal Service Labor-Management Relations Statute (92 Stat. 1214). Additionally, the National Federation of Federal Employees (NFFE) has requested a major policy statement as to the application of the official time provisions of section 7131(a) of the Statute (92 Stat. 1214) to all negotiations between an exclusive representative and an agency, regardless of whether such negotiations pertain to the negotiation or renegotiation of a basic collective bargaining agreement. AFGE has raised a similar issue in its request.

The Authority hereby determines, in conformity with 5 CFR 2410.3(a) (1978) and section 7135(b) of the Statute (92 Stat. 1215), as well as section 7105 of the Statute (92 Stat. 1196), that an interpretation of the Statute is warranted on the following:

(1) Whether employees who are on official time under section 7131 of the Statute while representing an exclusive representative in the negotiation of a collective bargaining agreement are entitled to payments from agencies for their travel and per diem expenses.

(2) Whether the official time provisions of section 7131(a) of the Statute encompass all negotiations between an exclusive representative and an agency, regardless of whether such negotiations pertain to the negotiation or renegotiation of a basic collective bargaining agreement.

Before issuing an interpretation on the above, the Authority, pursuant to 5 CFR 2410.6 (1978) and section 7135(b) of the Statute (92 Stat. 1215), solicits your views in writing. You are further invited to address the impact, if any, of section 7135(a)(1) of the Statute (92 Stat. 1215) on the above matters and to submit your views as to whether oral argument should be granted. To receive consideration, such views must be submitted to the Authority by the close of business on August 24, 1979.

Issued, Washington, D.C., July 13, 1979.

Federal Labor Relations Authority.

Ronald W. Haughton,

Chairman.

Henry B. Frazier III,

Member.

[FR Doc. 79-22440 Filed 7-19-79; 8:45 am]

BILLING CODE 6325-01-M

B 35

MOU number: 225-79-2001

Memorandum of Understanding

Between
The Environmental Protection Agency

and

The Food and Drug Administration

I. Purpose:

This Memorandum of Understanding establishes an agreement between the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) with regard to the control of direct and indirect additives to and substances in drinking water.

EPA and FDA agree:

A. That contamination of drinking water from the use and application of direct and indirect additives and other substances poses a potential public health problem;

B. That the scope of the additives problem in terms of the health significance of these contaminants in drinking water is not fully known;

C. That the possibility of overlapping jurisdiction between EPA and FDA with respect to control of drinking water additives has been the subject of Congressional as well as public concern;

D. That the authority to control the use and application of direct and indirect additives to and substances in drinking water should be vested in a single regulatory agency to avoid duplicative and inconsistent regulation;

E. That EPA has been mandated by Congress under the Safe Drinking Water Act (SDWA), as amended, to assure that the public is provided with safe drinking water;

F. That EPA has been mandated by Congress under the Toxic Substances Control Act (TSCA) to protect against unreasonable risks to health and the environment from toxic substances by requiring, inter alia, testing and necessary restrictions on the use, manufacture, processing, distribution, and disposal of chemical substances and mixtures;

G. That EPA has been mandated by Congress under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, to assure, inter alia, that when used properly, pesticides will perform their intended function without causing unreasonable adverse effects on the environment; and,

H. That FDA has been mandated by Congress under the Federal Food, Drug, and

B-36

Cosmetic Act (FFDCA), as amended, to protect the public from, inter alia, the adulteration of food by food additives and poisonous and deleterious substances.

It is the intent of the parties that:

A. EPA will have responsibility for direct and indirect additives to and other substances in drinking water under the SDWA, TSCA, and FIFRA; and,

B. FDA will have responsibility for water, and substances in water, used in food and for food processing and responsibility for bottled drinking water under the FFDCA.

II. Background:

A. FDA Legal Authority

"Food" means articles used for food or drink for man or other animals and components of such articles. (FFDCA Section 201(f)). Under Section 402, inter alia, a food may not contain any added poisonous or deleterious substance that may render it injurious to health, or be prepared, packed or handled under unsanitary conditions. Tolerances may be set, under Section 406, limiting the quantity of any substance which is required for the production of food or cannot be avoided in food. FDA has the authority under Section 409 to issue food additive regulations approving, with or without conditions, or denying the use of a "food additive." That term is defined in Section 201(s) to include any substance the intended use of which results or may reasonable be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food, if such substance is not generally recognized as safe.

In the past, FDA has considered drinking water to be a food under Section 201(f). However, both parties have determined that the passage of the SDWA in 1974 implicitly repealed FDA's authority under the FFDCA over water used for drinking water purposes. Under the express provisions of Section 410 of the FFDCA, FDA retains authority over bottled drinking water. Furthermore, all water used in food remains a food and subject to the provisions of the FFDCA. Water used for food processing is subject to applicable provisions of FFDCA. Moreover, all substances in water used in food are added substances subject to the provisions of the FFDCA, but no substances added to a public drinking water system before the water enters a food processing establishment will be considered a food additive.

B. EPA Legal Authority

The SDWA grants EPA the authority to control contaminants in drinking water which may have any adverse effect on the public health, through the establishment of maximum contaminant levels (MCLs) or treatment techniques, under Section 1412, which are applicable to owners and operators of public water systems. The expressed intent of the Act was to give EPA exclusive control over the safety of public water supplies. Public water systems may also be required by regulation to conduct monitoring for unregulated contaminants under Section 1445 and to issue public notification of such levels under Section 1414(c).

EPA's direct authority to control additives to drinking water apart from the existence of maximum contaminant levels or treatment techniques is limited to its emergency powers under Section 1431. However, Section 1442(b) of the Act authorizes EPA to "collect and make available information pertaining to research, investigations, and demonstrations with respect to providing a dependably safe supply of drinking water together with appropriate recommendations therewith."

TSCA gives EPA authority to regulate chemical substances, mixtures and under some circumstances, articles containing such substances or mixtures. Section 4 permits EPA

to require testing of a chemical substance or mixture based on possible unreasonable risk of injury to health or the environment, or on significant or substantial human or environmental exposure while Section 8 enables EPA to require submission of data showing substantial risk of injury to health or the environment, existing health and safety studies, and other data. For new chemical substances, and significant new uses of existing chemical substances, Section 5 requires manufacturers to provide EPA with pre-manufacturing notice. Under Section 6 the manufacture, processing, distribution, use, and disposal of a chemical substance or mixture determined to be harmful may be restricted or banned. Although Section 3(2)(B) of TSCA excludes from the definition of "chemical substance" food and food additives as defined under FFDCA, the implicit repeal by the SDWA of FDA's authority over drinking water enables EPA to regulate direct and indirect additives to drinking water as chemical substances and mixtures under TSCA.

The FIFRA requires EPA to set restrictions on the use of pesticides to assure that when used properly, they will not cause unreasonable adverse effects on the environment. EPA may require, inter alia labeling which specifies how, when, and where a pesticide may be legally used. In addition, EPA has, under Section 409 of the FFDCA, required FIFRA registrants at times to obtain a food additive tolerance before using a pesticide in or around a drinking water source. Such tolerances establish further restrictions on the use of a pesticide which are enforceable against the water supplier as well as the registrant of the pesticide.

III. Terms of Agreement:

A. EPA's responsibilities are as follows:

1. To establish appropriate regulations, and to take appropriate measures, under the SDWA and/or TSCA, and FIFRA, to control direct additives to drinking water (which encompass any substances purposely added to the water), and indirect additives (which encompass any substance which might leach from paints, coatings or other materials as an incidental result of drinking water contact), and other substances.

2. To establish appropriate regulations under the SDWA to limit the concentrations of pesticides in drinking water; the limitations on concentrations and types of pesticides in water are presently set by EPA through tolerances under Section 409 of the FFDCA.

3. To continue to provide technical assistance in the form of informal advisory opinions on drinking water additives under Section 1442(b) of the SDWA.

4. To conduct and require research and monitoring and the submission of data relative to the problem of direct and indirect additives in drinking water in order to accumulate data concerning the health risks posed by the presence of these contaminants in drinking water.

B. FDA's responsibilities are as follows:

1. To take appropriate regulatory action under the authority of the FFDCA to control bottled drinking water and water, and substances in water, used in food and for food processing.

2. To provide assistance to EPA to facilitate the transition of responsibilities, including:

a) To review existing FDA approvals in order to identify their applicability to additives in drinking water.

b) To provide a mutually agreed upon level of assistance in conducting literature searches related to toxicological decision making.

c) To provide a senior toxicologist to help EPA devise new procedures and protocols to be used in formulating advice on direct and indirect additives to drinking water.

IV. Duration of Agreement:

This Memorandum of Understanding shall continue in effect unless modified by mutual consent of both parties or terminated by either party upon thirty (30) days advance written notice to the other.

This Memorandum of Understanding will become effective on the date of the last signature.

Approved and Accepted for the Environmental Protection Agency

Signed by: Douglas P. Costle
Administrator
Environmental Protection Agency

Date: June 12, 1979

Approved and Accepted for the Food and Drug Administration

Signed by: Donald Kennedy
Administrator
Food and Drug Administration

Date: June 22, 1979

Domestic MOUs

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FDA Website Management Staff

§ 321. Definitions; Generally.

Archive

United States Statutes

Title 21. Food and Drugs

Chapter 9. FEDERAL FOOD, DRUG, AND COSMETIC ACT

Subchapter II. DEFINITIONS

Current through P.L. 111-290

§ 321. Definitions; Generally

For the purposes of this chapter-

(a) (1) The term "State", except as used in the last sentence of section **372 (a)** of this title, means any State or Territory of the United States, the District of Columbia, and the Commonwealth of Puerto Rico.

(2) The term "Territory" means any Territory or possession of the United States, including the District of Columbia, and excluding the Commonwealth of Puerto Rico and the Canal Zone.

(b) The term "interstate commerce" means

(1) commerce between any State or Territory and any place outside thereof, and

(2) commerce within the District of Columbia or within any other Territory not organized with a legislative body.

(c) The term "Department" means Department of Health and Human Services.

(d) The term "Secretary" means the Secretary of Health and Human Services.

(e) The term "person" includes individual, partnership, corporation, and association.

(f) The term "food" means

(1) articles used for food or drink for man or other animals,

(2) chewing gum, and

B 40

(3) articles used for components of any such article.

(g) (1) The term "drug" means

(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; and

(B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and

(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). A food or dietary supplement for which a claim, subject to sections **343 (r)(1)(B)** and **343 (r)(3)** of this title or sections **343 (r)(1)(B)** and **343 (r)(5)(D)** of this title, is made in accordance with the requirements of section **343 (r)** of this title is not a drug solely because the label or the labeling contains such a claim. A food, dietary ingredient, or dietary supplement for which a truthful and not misleading statement is made in accordance with section **343 (r)(6)** of this title is not a drug under clause (C) solely because the label or the labeling contains such a statement.

(2) The term "counterfeit drug" means a drug which, or the container or labeling of which, without authorization, bears the trademark, trade name, or other identifying mark, imprint, or device, or any likeness thereof, of a drug manufacturer, processor, packer, or distributor other than the person or persons who in fact manufactured, processed, packed, or distributed such drug and which thereby falsely purports or is represented to be the product of, or to have been packed or distributed by, such other drug manufacturer, processor, packer, or distributor.

(h) The term "device" (except when used in paragraph (n) of this section and in sections **331 (i)**, **343 (f)**, **352 (c)**, and **362 (c)** of this title) means an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is-

(1) recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them,

(2) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or

(3) intended to affect the structure or any function of the body of man or other animals, and

which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended

B 41

(dd) For purposes of sections **335a** and **335b** of this title, the term "drug product" means a drug subject to regulation under section **355**, **360b**, or **382** of this title or under section **262** of title 42.

(ee) The term "Commissioner" means the Commissioner of Food and Drugs.

(ff) The term "dietary supplement"-

(1) means a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients:

(A) a vitamin;

(B) a mineral;

(C) an herb or other botanical;

(D) an amino acid;

(E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or

(F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E);

(2) means a product that-

(A)

(i) is intended for ingestion in a form described in section **350 (c)(1)(B)(i)** of this title; or

(ii) complies with section **350 (c)(1)(B)(ii)** of this title;

(B) is not represented for use as a conventional food or as a sole item of a meal or the diet; and

(C) is labeled as a dietary supplement; and

(3) does-

(A) include an article that is approved as a new drug under section **355** of this title or licensed as a biologic under section **262** of title 42 and was, prior to such approval, certification, or license, marketed as a dietary supplement or as a food unless the Secretary has issued a regulation, after notice and comment, finding that the article, when used as or in a dietary supplement under the conditions of use and dosages set forth in the labeling for such dietary supplement, is unlawful under section **342 (f)** of

B 42

this title; and

(B) not include-

(i) an article that is approved as a new drug under section **355** of this title, certified as an antibiotic under section **357** of this title, or licensed as a biologic under section **262** of title 42, or

(ii) an article authorized for investigation as a new drug, antibiotic, or biological for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public,

which was not before such approval, certification, licensing, or authorization marketed as a dietary supplement or as a food unless the Secretary, in the Secretary's discretion, has issued a regulation, after notice and comment, finding that the article would be lawful under this chapter.

Except for purposes of paragraph (g) and section 350f of this title, a dietary supplement shall be deemed to be a food within the meaning of this chapter.

(gg) The term "processed food" means any food other than a raw agricultural commodity and includes any raw agricultural commodity that has been subject to processing, such as canning, cooking, freezing, dehydration, or milling.

(hh) The term "Administrator" means the Administrator of the United States Environmental Protection Agency.

(ii) The term "compounded positron emission tomography drug"-

(1) means a drug that-

(A) exhibits spontaneous disintegration of unstable nuclei by the emission of positrons and is used for the purpose of providing dual photon positron emission tomographic diagnostic images; and

(B) has been compounded by or on the order of a practitioner who is licensed by a State to compound or order compounding for a drug described in subparagraph (A), and is compounded in accordance with that State's law, for a patient or for research, teaching, or quality control; and

(2) includes any nonradioactive reagent, reagent kit, ingredient, nuclide generator, accelerator, target material, electronic synthesizer, or other apparatus or computer program to be used in the preparation of such a drug.

343

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

DEC 21 2000

The Honorable Ken Calvert
Chairman
Subcommittee on Energy and Environment
Committee on Science
House of Representatives
Washington, D.C. 20515-6301

Dear Mr. Chairman:

Thank you for the letter of May 8, 2000, to Dr. Jane E. Henney, Commissioner of Food and Drugs, regarding the use of fluoride in drinking water and drug products. We apologize for the delay in responding to you.

We have restated each of your questions, followed by our response.

1. **If health claims are made for fluoride-containing products (e.g. that they reduce dental caries incidence or reduce pathology from osteoporosis), do such claims mandate that the fluoride-containing product be considered a drug, and thus subject the product to applicable regulatory controls?**

Fluoride, when used in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or animal, is a drug that is subject to Food and Drug Administration (FDA) regulation. FDA published a final rule on October 6, 1995, for anticaries drug products for over-the-counter (OTC) human use (copy enclosed). This rule establishes the conditions under which OTC anticaries drug products are generally recognized as safe and effective and not misbranded. The rule has provisions for active ingredients, packaging conditions, labeling, and testing procedures that are required by manufacturers in order to market anticaries products. A new drug application (NDA) may be filed for a product containing fluoride that does not meet the provisions stated in the final rule. As you know, the Environmental Protection Agency regulates fluoride in the water supply.

B 44

2. Are there any New Drug Applications (NDA) on file, that have been approved, or that have been rejected, that involve a fluoride-containing product (including fluoride-containing vitamin products) intended for ingestion with the stated aim of reducing dental caries? If any such NDA's have been rejected, on what grounds were they rejected? If any such NDA have been approved, please provide the data on safety and efficacy that FDA found persuasive.

No NDAs have been approved or rejected for fluoride drugs meant for ingestion. Several NDAs have been approved for fluoride topical products such as dentifrices and gels. Fluoride products in the form of liquid and tablets meant for ingestion were in use prior to enactment of the Kefauver-Harris Amendments (Drug Amendments of 1962) to the Food, Drug, and Cosmetic Act in which efficacy became a requirement, in addition to safety, for drugs marketed in the United States (U.S.). Drugs in use prior to 1962 are being reviewed under a process known as the drug efficacy study implementation (DESI). The DESI review of fluoride-containing products has not been completed.

3. Does FDA consider dental fluorosis a sign of over exposure to fluoride?

Dental fluorosis is indicative of greater than optimal ingestion of fluoride. In 1988, the U.S. Surgeon General reported that dental fluorosis, while not a desirable condition, should be considered a cosmetic effect rather than an adverse health effect. Surgeon General M. Joycelyn Elders reaffirmed this position in 1994.

4. Does FDA have any action-level or other regulatory restriction or policy statement on fluoride exposure aimed at minimizing chronic toxicity in adults or children?

The monograph for OTC anticaries drug products sets acceptable concentrations for fluoride dentifrices, gels and rinses (all for topical use only). This monograph also describes the acceptable dosing regimens and labeling including warnings and directions for use. FDA's principal safety concern regarding fluoride in OTC drugs is the incidence of fluorosis in

children. Children under two years of age do not have control of their swallowing reflex and do not have the skills to expectorate toothpaste properly. Young children are most susceptible to mild fluorosis as a result of improper use and swallowing of a fluoride toothpaste. These concerns are addressed in the monograph by mandating maximum concentrations, labeling that specifies directions for use and age restrictions, and package size limits.

Thanks again for contacting us concerning this matter. If you have further questions, please let us know.

Sincerely,

Melinda K. Plaisier
Associate Commissioner
for Legislation

Enclosure
"Final Rule/Federal Register - October 6, 1995
Over-the-Counter Anticaries Drug Products"

Web site administrator's note:
To perform query to access this document
Enter: http://www.access.gpo.gov/su_docs/aces/aces140.html
Enter: checkmark for 1995 Volume 60
Enter: On: 10/06/95
Enter: Search terms: anticaries

Fluorosilicic Acid

Fluorosilicic Acid (Hydrofluorosilicic Acid, HFS, FSA)

Technical Data Sheet

CHEMICAL ANALYSIS	SPECIFICATION	TYPICAL ANALYSIS
H_2SiF_6, %	23-25	23.5
Heavy Metals (as Pb), %		< 0.02
HF, %	1.0 max	0.5
Color, APHA	100 max	< 20
P_2O_5, %		< 0.2

Product meets ANSI/AWWA Standard B703-06, and is certified by NSF International or Classified by UL to ANSI/NSF Standard 60. Maximum use level for potable water treatment is 6.0 mg/L.

PHYSICAL PROPERTIES

Physical Description	Aqueous solution, water white to straw-yellow, corrosive acid, irritating to skin and having pungent odor.
Molecular Weight	144.08
Specific Gravity 23% solution @ 75°F	1.212
Boiling Point of Aqueous 23% Solution	221°F (Decomposes)
Freezing Point of Aqueous 23% Solution	5°F (approx.)
Freezing Point of Aqueous 25% Solution	-4°F
pH of 1%, H_2SiF_6	1.2

CONTAINERS

Tank truck, rubber or plastic-lined	40,000 lb (approx.)
Tank car, rubber or plastic-lined	196,000 lb net (approx.)

DOT AND FREIGHT DESCRIPTION

Hazardous Material Description	Fluorosilicic acid
Haz. Mat. Class, I.D.#, Packaging Group	8, UN 1778, PG II
Freight Classification	Hydrofluorosilicic Acid
Principal CAS Number	16961-83-4
RQ	None
Placard	Corrosive
Label	Corrosive

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CGR#3323 HFS-0205 Revised 0707

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SOLVAY Fluorides

SOLVAY

B47

Fluorosilicic Acid

Fluorosilicic Acid (Hydrofluorosilicic Acid, HFS, FSA)

Technical Data Sheet

Use in public Water Treating Plants:

The reduction in dental caries by adjusting the fluoride content of public water supplies is a matter of common knowledge today, half a century following the first installation in Grand Rapids, Michigan. Approximately 170 million people in over three thousand communities are now drinking fluoride-treated water from water purification plants where fluoridation is currently practiced. Fluoridation is concerned with the controlled introduction to water of the fluoride ion. Other materials in the fluoride compound simultaneously introduced into the water with the fluoride ions are carriers which provide no benefits and are nontoxic. The addition of one part per million of fluoride requires that the product be soluble, of definite concentration and have high purity standards. In conformity with the American Water Works Association standard B703-94, the term fluorosilicic acid has replaced the more technical designation of hydrofluosilicic acid. After the original work with sodium fluoride proved the effectiveness of fluoride on tooth health and a broad fluoridation program was envisaged, new sources of fluoride and economics of their use were investigated. Fluorosilicic acid is a high purity source of fluoride. It is simpler to use than any other chemical approved for water fluoridation purposes, primarily because it is a liquid and can therefore be accurately measured and fed with a minimum of equipment. In contrast to powdered or granular chemicals, it presents no dust problems, no measuring problems and handling requires a minimum of labor. Today most of the large cities and many small ones are fluoridating with fluorosilicic acid. It is readily available in tank cars or tank trucks and can also be supplied in 15-gallon carboys and 55-gallon drums. The addition of fluorosilicic acid to a water supply can be readily controlled to give a total fluoride (F) level of one part per million which has been established as effective for reducing tooth decay. It should be used in accordance with procedures approved by each state's department of health.

Acid Characteristics:

Fluorosilicic acid is a transparent, clear to straw-colored, corrosive liquid having the chemical formula of H_2SiF_6. It is manufactured in modern rubber-lined equipment producing an acid of high commercial purity. Commercial water solutions of the acid are available, having concentration of between 23% and 25% H_2SiF_6. Fluorosilicic acid is generally believed not to exist in the vapor phase, but only in solution. Upon vaporizing, it decomposes into hydrofluoric acid (HF) and silicon tetrafluoride. This equilibrium exists at the surface of strong solutions of fluorosilicic acid and if stored in glass containers, the small concentration of hydrofluoric acid may very slowly attack the glass above the solution level. For this reason, it is generally shipped in polyethylene containers rather than glass carboys. A 23% fluorosilicic acid-water solution weighs 10.1 pounds per gallon at 75°F, and has a fluoride (F) content of 18.20%.

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B48

Fluorosilicic Acid (Hydrofluorosilicic Acid, HFS, FSA)

Technical Data Sheet

Installation:
In a typical large plant installation, rubber-lined vented storage tanks are usually mounted outside the building with the tanks ranging in size from 4,500 to 6,500 gallon capacities. These tanks, equipped with recording level gauges, feed the acid through plastic piping or tubing to the dosage unit. Feeding is regulated by controlled volume pumps. Metering is used for accurate flow records. Fluorosilicic acid may be handled in rubber-lined, saran or other available corrosive-resistant equipment as suggested below:

Pipes and lines	-	rubber, saran or polyethylene
Pumps	-	Lucite, saran or Hastelloy
Valves	-	rubber-lined or polyethylene-lined
Tanks	-	rubber-lined, saran or polyethylene-lined

Acid should be pumped by positive diaphragm proportioning pumps.

Operation procedure:
The drum or drums of fluorosilicic acid should be mounted on a platform of sufficient size and capacity to permit weighing the amount used each day. Proportioning pumps deliver an accurate volume, but for small pumping rates, the dosage may be more satisfactorily regulated by periodic weighing of the drum. Whenever a drum of fluorosilicic acid is replaced on the scale, the time and weight should be recorded in the daily operating log. Whenever dosage is changed to a varying pumpage, the time and feeder setting should be recorded in the daily log.

CGR#3323 HFS-0205 Revised 0707

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B 49

SHANGHAI MINTCHEM DEVELOPMENT CO., LTD

Specification Sheet

Sodium Fluoride

Physical Properties:

Formula	**Na F**	**Molecular Weight**	**41.99**	**CAS NO**	**7681-49-4**
U.N-NO	**1690**	**Class**	**6.1**	**H.S-NO**	**2826110010**

Character: White crystal or powder. Relative Density 2.558. It's odorless. Soluble in water and HF. Insoluble in ethanol. Mellting point 993ºC and boiling point 1695ºC. Non flammable but toxic.

Chemical Parameters:

NO.	Technological Specification	Granular Standard%		Powder Standard%	
1	NaF purity	98.5%min		98.5%min	
2	Sodium Carbonate	0.5%max.		0.5%max.	
3	Na2SiF6	1.5%max		1.5%max	
4	Silicon Dioxide	0.5%max.		0.5%max.	
5	Sulphate	0.3%max.		0.3%max.	
6	HF	0.1%max,		0.1%max,	
7	H2O(moisture)	0.5%max.		0.5%max.	
8	Heavy Metal(As Pb)	0.04%max.		0.04%max.	
9	Available Fluoride	43.8%min.		43.8%min.	
10	Water Insoluble matter	0.6%max.		0.6%max.	
11	Particle Size	-20 mesh	98%min	+80 mesh	4 % max
12		+100 mesh	50%min	+200 mesh	25 % max
13		-325 mesh			

APPLICATION: It is mainly used as fluxing agent, timber preservative and water treatment etc.

PACKAGE: Packing in plastic weaved bag 25kg each. TRANSPORTATION: DG, Class 6.1, UN 1690

MANUFACTURER: SHANGHAI MINTCHEM DEVELOPMENT CO., LTD

上海办 **Shanghai Office**

上海市浦东新区牡丹路 89 弄 4 号 602 室
R602,4#,89Nong, Mudan Road Pudong
Shanghai, China
Tel: 0086 21 6845 1592
Fax: 0086 21 6845 0923

...www.mintchem.com...

长沙办 **Changsha Office**

长沙市劳动西路 108 号长沙市化工研究所 HX 楼 707 室
HX707 ,108# Laodong Road West Changsha,
Hunan,China
Tel: 0086 731 8552 9244/8552 9245/ 8552 9246
Fax: 0086 731 8551 8 167/8553 0 767

B-50

mid 1980's[7] (Evans R.W, Stamm JW., 1991). Across all age groups more than 90% of fluorosis cases were very mild or mild. (Evans R.W, Stamm JW., 1991). The study did not include measures of fluoride intake. Concurrently, dental caries prevalence did not increase. (Lo ECM *et al,* 1990). Although not fully generalizable to the current U.S. context, these findings, along with those from the 1986–87 survey of U.S. schoolchildren, suggest that risk of fluorosis can be reduced and caries prevention maintained toward the lower end (*i.e.,* 0.7 mg/L) of the 1962 USPHS recommendations for fluoride concentrations for community water systems.

Relationship of fluid intake and ambient temperature among children and adolescents in the United States:

The 1962 USPHS recommendations stated that community drinking water should contain 0.7–1.2 mg/L [ppm] fluoride, depending on the ambient air temperature of the area. These temperature-related guidelines were based on studies conducted in two communities in California in the early 1950's. Findings indicated that a lower fluoride concentration was appropriate for communities in warmer climates because children drank more tap water on warm days (Galagan DJ, 1953; Galagan DJ and Vermillion JR, 1957; Galagan DJ *et al,* 1957). Social and environmental changes, including increased use of air conditioning and more sedentary lifestyles, have occurred since the 1950's, and thus, the assumption that children living in warmer regions drink more tap water than children in cooler regions may no longer be valid.

Studies conducted since 2001 suggest that fluid intake in children does not increase with increases in ambient air temperature (Sohn W, *et al,* 2001; Beltrán-Aguilar ED, *et al,* 2010b). One study conducted among children using nationally representative data from 1988 to 1994 did not find an association between fluid intake and ambient air temperature (Sohn W, *et al,* 2001). A similar study using nationally representative data from 1999 to 2004 also found no association between fluid intake and ambient temperature among children or adolescents (Beltrán-Aguilar ED, *et al,* 2010b). These recent findings demonstrating a lack of an association between fluid intake among children and adolescents and ambient temperature support use of a single target concentration for community water fluoridation in all temperature zones of the United States.

Conclusions

HHS recommends an optimal fluoride concentration of 0.7 mg/L for community water systems based on the following information:

- Community water fluoridation is the most cost-effective method of delivering fluoride for the prevention of tooth decay;
- In addition to drinking water, other sources of fluoride exposure have contributed to the prevention of dental caries and an increase in dental fluorosis prevalence;
- Significant caries preventive benefits can be achieved and risk of fluorosis reduced at 0.7 mg/L, the lowest concentration in the range of the USPHS recommendation.
- Recent data do not show a convincing relationship between fluid intake and ambient air temperature. Thus, there is no need for different recommendations for water fluoride concentrations in different temperature zones.

Surveillance Activities

CDC and the National Institute of Dental and Craniofacial Research (NIDCR), in coordination with other Federal agencies, will enhance surveillance of dental caries, dental fluorosis, and fluoride intake with a focus on younger populations at higher risk of fluorosis to obtain the best available and most current information to support effective efforts to improve oral health.

Process

The U.S. Department of Health and Human Services (HHS) convened a Federal inter-departmental, inter-agency panel of scientists (Appendix A) to review scientific evidence related to the 1962 USPHS Drinking Water Standards related to recommendations for fluoride concentrations in drinking water in the United States and to update these proposed recommendations. Panelists included representatives from the Centers for Disease Control and Prevention, the National Institutes of Health, the Food and Drug Administration, the Agency for Healthcare Research and Quality, the Office of the Assistant Secretary for Health, the U.S. Environmental Protection Agency, and the U.S. Department of Agriculture. The panelists evaluated existing recommendations for fluoride in drinking water, systematic reviews of the risks and benefits from fluoride in drinking water, the epidemiology of dental caries and fluorosis in the U.S., and current data on fluid intake in children, aged 0 to 10 years, across temperature gradients in the U.S. Conclusions were reached and are summarized along with their rationale in this proposed guidance document. This guidance will be advisory, not regulatory, in nature. Guidance will be submitted to the **Federal Register** and will undergo public and stakeholder comment for 30 days, after which HHS will review comments and consider changes.

Dated: January 7, 2011.

Kathleen Sebelius,

Secretary.

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[7] Fluorosis prevalence ranged from 64% (SE = 4.1) to 47% (SE = 4.5) based on the upper right central incisor only.

B 51

STATE OF WASHINGTON

WASHINGTON STATE BOARD OF HEALTH

PO Box 47990 • Olympia, Washington 98504-7990

November 16, 2010

Mr. William Osmunson, DDS, MPH, President
Washington Action for Safe Water
1418 – 112th Ave NE, Suite 200
Bellevue, WA 98004

Dear Dr. Osmunson:

This letter provides formal notice that the Washington State Board of Health has denied your petition for rule making received on October 7, 2010 to add an intent statement in two places in WAC 246-290-460, regarding water fluoridation. The suggested statement was "with the intent to prevent dental caries." This was the fifth petition for rule making you submitted to the Board this year regarding this rule.

The Board's intent for setting an "optimal" fluoride concentration in WAC 246-290-460 is part of its requirement to "adopt rules for group A public water systems...to assure safe and reliable public drinking water and to protect the public health" under RCW 43.20.050(2)(a). The Board follows guidelines of the Centers for Disease Control and Prevention (CDC) regarding setting an appropriate level of fluoride in drinking water if the directors of a water system decide to fluoridate under the authority of RCW 57.08.012. The CDC promotes community water fluoridation as one of the ten great public health achievements of the twentieth century. It says fluoridation is the single most effective public health measure to prevent tooth decay. The Board supports this and other positions of the CDC. The Board considers it self evident that the purpose of water fluoridation is to help prevent tooth decay. The Board does not consider it efficient use of public resources to initiate and complete a rule making process to add to the rule the language requested by the petitioner.

The Board handled your request as a petition for rule making under RCW 34.05.330 and Board Policy 2005-001, Responding to Petitions for Rule Making. The statute requires the Board to respond within 60 days of receipt. RCW 34.05.330(3) allows a person to appeal a petition's denial to the Governor within 30 days. The Board's policy allows the Board Chair to respond to a petition for rule making without the petition being placed on a meeting agenda for full Board consideration. If you have questions about this decision, please contact Craig McLaughlin, Executive Director of the Board, at 360-236-4106 or craig.mclaughlin@doh.wa.gov.

Sincerely,

Keith Higman
Chair

cc: Michelle Davis, Department of Health
Gregg Grunenfelder, Department of Health
State Board of Health Members

B 52

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

August 17, 2001 / Vol. 50 / No. RR-14

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WEEKLY REPORT

Recommendations and Reports

Recommendations for Using Fluoride to Prevent and Control Dental Caries in the United States

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30333

B59

Recommendations for Using Fluoride to Prevent and Control Dental Caries in the United States

Summary

Widespread use of fluoride has been a major factor in the decline in the prevalence and severity of dental caries (i.e., tooth decay) in the United States and other economically developed countries. When used appropriately, fluoride is both safe and effective in preventing and controlling dental caries. All U.S. residents are likely exposed to some degree to fluoride, which is available from multiple sources. Both health-care professionals and the public have sought guidance on selecting the best way to provide and receive fluoride. During the late 1990s, CDC convened a work group to develop recommendations for using fluoride to prevent and control dental caries in the United States. This report includes these recommendations, as well as a) critical analysis of the scientific evidence regarding the efficacy and effectiveness of fluoride modalities in preventing and controlling dental caries, b) ordinal grading of the quality of the evidence, and c) assessment of the strength of each recommendation.

Because frequent exposure to small amounts of fluoride each day will best reduce the risk for dental caries in all age groups, the work group recommends that all persons drink water with an optimal fluoride concentration and brush their teeth twice daily with fluoride toothpaste. For persons at high risk for dental caries, additional fluoride measures might be needed. Measured use of fluoride modalities is particularly appropriate during the time of anterior tooth enamel development (i.e., age <6 years).

The recommendations in this report guide dental and other health-care providers, public health officials, policy makers, and the public in the use of fluoride to achieve maximum protection against dental caries while using resources efficiently and reducing the likelihood of enamel fluorosis. The recommendations address public health and professional practice, self-care, consumer product industries and health agencies, and further research. Adoption of these recommendations could further reduce dental caries in the United States and save public and private resources.

INTRODUCTION

Dental caries (i.e., tooth decay) is an infectious, multifactorial disease afflicting most persons in industrialized countries and some developing countries (*1*). Fluoride reduces the incidence of dental caries and slows or reverses the progression of existing lesions (i.e., prevents cavities). Although pit and fissure sealants, meticulous oral hygiene, and appropriate dietary practices contribute to caries prevention and control, the most effective and widely used approaches have included fluoride use. Today, all U.S. residents are exposed to fluoride to some degree, and widespread use of fluoride has been a major factor in the decline in the prevalence and severity of dental caries in the United States and other economically developed countries (*1*). Although this decline is a major public

B54

Fluoridated Drinking Water and Processed Beverages and Food

Fluoridated drinking water contains a fluoride concentration effective for preventing dental caries; this concentration can occur naturally or be reached through water fluoridation, which is the controlled addition of fluoride to a public water supply. When fluoridated water is the main source of drinking water, a low concentration of fluoride is routinely introduced into the mouth. Some of this fluoride is taken up by dental plaque; some is transiently present in saliva, which serves as a reservoir for plaque fluoride; and some is loosely held on the enamel surfaces (*76*). Frequent consumption of fluoridated drinking water and beverages and food processed in fluoridated areas maintains the concentration of fluoride in the mouth.

Estimates of fluoride intake among U.S. and Canadian adults have ranged from ≤1.0 mg fluoride per day in nonfluoridated areas to 1–3 mg fluoride per day in fluoridated areas (*77–80*). The average daily dietary fluoride intake for both children and adults in fluoridated areas has remained relatively constant for several years (*11*). For children who live in optimally fluoridated areas, this average is approximately 0.05 mg/kg/day (range: 0.02–0.10); for children who live in nonfluoridated areas, the average is approximately half (*11*). In a survey of four U.S. cities with different fluoride concentrations in the drinking water (range: 0.37–1.04 ppm), children aged 2 years ingested 0.41–0.61 mg fluoride per day and infants aged 6 months ingested 0.21–0.54 mg fluoride per day (*81,82*).

In the United States, water and processed beverages (e.g., soft drinks and fruit juices) can provide approximately 75% of a person's fluoride intake (*83*). Many processed beverages are prepared in locations where the drinking water is fluoridated. Foods and ingredients used in food processing vary in their fluoride content (*11*). As consumption of processed beverages by children increases, fluoride intake in communities without fluoridated water will increase whenever the water source for the processed beverage is fluoridated (*84*). In fluoridated areas, dietary fluoride intake has been stable because processed beverages have been substituted for tap water and for beverages prepared in the home using tap water (*11*).

A study of Iowa infants estimated that the mean fluoride intake from water during different periods during the first 9 months of life, either consumed directly or added to infant formula or juice, was 0.29–0.38 mg per day, although estimated intake for some infants was as high as 1.73 mg per day (*85*). As foods are added to an infant's diet, replacing some of the formula prepared with fluoridated water, the amount of fluoride the infant receives typically decreases (*86*). The Iowa study also reported that infant formula and processed baby food contained variable amounts of fluoride. Since 1979, U.S. manufacturers of infant formula have voluntarily lowered the fluoride concentration of their products, both ready-to-feed and concentrates, to <0.3 ppm fluoride (*87*).

Drinking Water

Community Water. During the 1940s, researchers determined that 1 ppm fluoride was the optimal concentration in community drinking water for climates similar to the Chicago area (*88,89*). This concentration would substantially reduce the prevalence of dental caries, while allowing an acceptably low prevalence (i.e., 10%–12%) of very mild and mild enamel fluorosis and no moderate or severe enamel fluorosis. Water fluoridation for caries control began in 1945 and 1946, when the fluoride concentration was

B.55

U.S. Food and Drug Administration

Regulatory Information

Significant Amendments to the FD&C Act

Significant Amendments to the FD&C Act:

Since 1980, listed chronologically; date shown is when the Public Law was approved. "Summary" indicates link to a summary of the law; other links are to full text. Provisions of these Public Laws are incorporated into the FD&C Act.

- Infant Formula Act of 1980 (summary)[1]
  Public Law (PL) 96-359 (Oct. 26, 1980)
- Orphan Drug Act[2]
  PL 97-414 (Jan. 4, 1983)
- Drug Price Competition and Patent Term Restoration Act of 1984 (summary)[3]
  PL 98-417 (Sept. 24, 1984)
- Prescription Drug Marketing Act of 1987[4]
  PL 100-293 (Apr. 22, 1988)
- Generic Animal Drug and Patent Term Restoration Act of 1988 (summary)[5]
  PL 100-670 (Nov. 16, 1988)
- Nutrition Labeling and Education Act of 1990 (summary)[6]
  PL 101-535 (Nov. 8, 1990)
- Safe Medical Devices Act of 1990 (summary)[7]
  PL 101-629 (Nov. 28, 1990)
- Medical Device Amendments of 1992 (summary)[8]
  PL 102-300 (June 16, 1992)
- Prescription Drug Amendments of 1992; Prescription Drug User Fee Act of 1992[9]
  PL 102-571 (Oct. 29, 1992)
- Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994[10]
  PL 103-396 (Oct. 22, 1994)
- Dietary Supplement Health and Education Act of 1994[11]
  PL 103-417 (Oct. 25, 1994)
- FDA Export Reform and Enhancement Act of 1996[12]
  PL 104-134 (April 26, 1996)
- Food Quality Protection Act of 1996[13]
  PL 104-170 (Aug. 3, 1996)
- Animal Drug Availability Act of 1996[14]
  PL 104-250 (Oct. 9, 1996)
- Food and Drug Administration Modernization Act (FDAMA) of 1997[15]
  PL 105-115 (Nov. 21, 1997)
- Best Pharmaceuticals for Children Act[16]
  PL 107-109 (Jan. 4, 2002)
- Medical Device User Fee and Modernization Act (MDUFMA) of 2002[17]
  PL 107-250 (Oct. 26, 2002)
- Animal Drug User Fee Act of 2003[18]
  PL 108-130 (Nov. 18, 2003)
- Pediatric Research Equity Act of 2003[19]
  PL 108-155 (Dec. 3, 2003)
- Minor Use and Minor Species Animal Health Act of 2004[20]
  PL 108-282 (Aug. 2, 2004)
- Dietary Supplement and Nonprescription Drug Consumer Protection Act[21]
  PL 109-462 (Dec. 22, 2006)
- Food and Drug Administration Amendments Act (FDAAA) of 2007[22]
  PL 110-85 (Sept. 27, 2007)
- Family Smoking Prevention and Tobacco Control Act (Public Law 111-31)[23]
  PL 111-31 (June 22, 2009)
- FDA Food Safety Modernization Act[24]
  PL 111-353 (Jan. 4, 2011)

Links on this page:

1. http://thomas.loc.gov/cgi-bin/bdquery/z?d096:HR06940:@@@L|TOM:/bss/d096query.html|#summary
2. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/OrphanDrugAct/default.htm

B56

3. http://thomas.loc.gov/cgi-bin/bdquery/z?d098:SN01538:@@@D&summ2=m&|TOM:/bss/d098query.html|
4. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/PrescriptionDrugMarketingActof1987/default.htm
5. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm147135.htm
6. http://thomas.loc.gov/cgi-bin/bdquery/z?d101:HR03562:@@@D&summ2=3&|TOM:/bss/d101query.html|
7. http://thomas.loc.gov/cgi-bin/bdquery/z?d101:HR03095:@@@D&summ2=1&|TOM:/bss/d101query.html|
8. http://thomas.loc.gov/cgi-bin/bdquery/z?d102:SN02783:@@@D&summ2=m&|TOM:/bss/d102query.html|
9. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/PrescriptionDrugAmendmentsof1992PrescriptionDrugUserFeeActof1992/default.htm
10. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/AnimalMedicinalDrugUseClarificationActAMDUCAof1994/default.htm
11. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148003.htm
12. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148005.htm
13. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148008.htm
14. http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=104_cong_public_laws&docid=f:publ250.104
15. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FDAMA/default.htm
16. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148011.htm
17. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/MedicalDeviceUserFeeandModernizationActMDUFMAof2002/default.htm
18. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/AnimalDrugUserFeeActof2003/default.htm
19. http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=108_cong_public_laws&docid=f:publ155.108
20. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/MinorUseandMinorSpeciesAnimalHealthActof2004/default.htm
21. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm148035.htm
22. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FoodandDrugAdministrationAmendmentsActof2007/default.htm
23. http://www.gpo.gov/fdsys/pkg/PLAW-111publ31/pdf/PLAW-111publ31.pdf
24. /RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/ucm244718.htm

857

NOV 21 2014

Dear Ms. McElheney:

Thank you for your correspondence concerning fluoridation of drinking water. Your letter requests that I take a number of actions related to fluoridation. These include instructing the Food and Drug Administration (FDA) to advise fluoridation manufacturers to submit New Drug Applications; instructing the Centers for Disease Control and Prevention (CDC) to stop "promotion. . . of any and all drugs, including the ingestion of fluoride products, not FDA CDER approved"; sponsoring a review of fluoride's neurotoxicity by the National Research Council; and supporting a prospective randomized control trial of the effectiveness of ingesting hydrofluorosilicic acid.

For nearly 70 years, community water fluoridation (CWF) has been a safe and healthy way to effectively prevent tooth decay. CDC has recognized water fluoridation as one of ten great public health achievements of the 20th century. CDC works with national partners, states, communities, and water operators to ensure that the U.S. population has access to optimally fluoridated water to prevent tooth decay.

However, fluoride ingestion while teeth are developing can result in a range of visually detectable changes in the tooth enamel, called dental fluorosis. The prevalence of mild to moderate dental fluorosis in the United States has increased in recent years. Fluoride in drinking water is one of several available fluoride sources. In 2011, the Department of Health and Human Services (HHS) proposed that the recommended level of fluoride in drinking water be set at 0.7 mg/L. This will reduce the chance for children's teeth to develop dental fluorosis, while still preventing tooth decay. The previous U.S. Public Health Service recommendations for fluoride levels ranged from 0.7mg/L to 1.2 mg/L, depending on average maximum regional air temperature. The new recommendation is based on recent findings that in the U.S., outdoor temperature does not determine water intake.

HHS expects that the final recommendations to reduce the optimal fluoride level will be publicly available soon. CDC, in collaboration with the National Institute of Dental and Craniofacial Research (NIDCR), will monitor the impact of these changes through enhanced surveillance of dental caries (tooth decay) and dental fluorosis in the National Health and Nutrition Examination Survey (NHANES).

Your specific requests are addressed below.

Instruct FDA CDER to no longer defer regulatory action. FDA CDER to send a letter to fluoridation manufacturers advising them to make FDA CDER NDA (New Drug Application) as required by Congress in the US FD&C Act.

FDA has provided the following information regarding your request:

> *FDA has determined that Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the FD&C Act. Instead, Congress intended that the U.S. Environmental Protection Agency (EPA) regulate fluoride in public drinking water as a potential contaminant under the Safe Drinking Water Act of 1974 (SDWA), Public Law No. 93-523, 88 Stat. 1660 (codified as amended at 42 U.S.C. 300f et seq.) to protect against adverse health effects, and that within the limits thus set by EPA, state and local governments be permitted, but not required, to fluoridate public drinking water to help prevent dental caries. Thus, FDA does not require NDAs for fluoridated public drinking water.*

Instruct the CDC to stop the promotion (internet and education) of any and all drugs, including the ingestion of fluoride products, not FDA CDER approved.

Section 317M of the Public Health Service Act, codified at 42 U.S.C. § 247b-14, authorizes the Secretary of HHS, acting through the Director of the CDC, to make grants to States and Indian tribes for the purpose of increasing the resources available for community water fluoridation. This includes funds to develop educational materials on the benefits of fluoridation. CDC's Division of Oral Health leads an effort to improve the oral health of the nation and reduce inequalities in oral health. This includes encouraging the use of proven strategies to prevent oral disease, such as the effective use of fluoride products and community water fluoridation.

Sponsor a review of the scientific evidence on fluoride's neurotoxicity by the National Academy of Science's National Research Council. The review should include studies listed at www.FluorideAlert.org/issues/health/brain.

The NRC reviewed the toxicity of fluoride as recently as 2006, when it reviewed the Environmental Protection Agency's drinking water standard for fluoride as a contaminant. (See *Fluoride in Drinking Water: A Scientific Review of EPA's Standards.*) More recently and of more relevance to community water fluoridation is the systematic review undertaken by the Community Preventive Services Task Force (Task Force) in 2013. The Task Force is an independent, nonfederal, unpaid panel of public health and prevention experts that provides evidence-based findings and recommendations about community preventive services, programs, and policies to improve health. Its members represent a broad range of research, practice, and policy expertise in community preventive services, public health, health promotion, and disease prevention. In its report, *Preventing Dental Caries: Community Water Fluoridation*, the Task Force noted, "Overall, the body of evidence indicates that Community Water Fluoridation is an effective intervention for reducing caries at the population level. At the optimal fluoride concentration, associated risks are predominantly the milder forms of fluorosis that are only detectable under clinical examination." The report further stated, "In addition, there is no evidence that CWF (Community Water Fluoridation) results in severe dental fluorosis."

Sponsor a quality published independent prospective randomized controlled trial (RTC), of the effectiveness of ingesting hydrofluorosilicic acid (fluoridation), including blood serum and urine concentrations of fluoride.

As stated above, the effectiveness and safety of community water fluoridation was reaffirmed by the Community Preventive Services Task Force in 2013 following a systematic evidence review. Studies on the effectiveness of adjusting fluoride in community water to the optimal concentration cannot be designed as randomized clinical trials. Random allocation of study subjects is not possible when a community begins to fluoridate the water because all residents receiving community water have access to and are exposed to this source of fluoride. Furthermore, clinical studies cannot be conducted double-blind because both study subjects and researchers usually know whether a community's water has been fluoridated. In addition, it would not be possible to find control subjects with no fluoride exposure because fluorides are ubiquitous in the environment.

Although I am not able to fulfill your requests, I appreciate the information you provided to me and my staff. I will keep your concerns in mind as HHS continues to consider community water fluoridation.

A copy of this response is being shared with Dr. Hirzy, Mr. Nidel, Dr. Connett, Ms. Smith, and Dr. Osmunson.

Sincerely,

Wanda K. Jones, DrPH
Principal Deputy Assistant Secretary for Health

B 60

Jill McElheney
Chris Nidel, Nidel Law 1615 New Hampshire Ave., NW, Washington, DC 20009. 202-558-2030
Bill Hirzy PhD Fluoride Action Network
Paul Connett PhD President, Fluoride Action Network
Bill Osmunson DDS, MPH Comprehensive Cosmetic Dentist 425.466.0100
54 Ponder Point, Sandpoint, Idaho 83864 bill@teachingsmiles.com

September 4, 2014

Wanda Jones
Jonathan Beeton
Office of the Assistant Secretary for Health
U.S. Department of Health and Human Services
Sandra.Howard@HHS.GOV
202-690-7778

For the health and safety of the public:

1. Instruct FDA CDER to no longer defer regulatory action. FDA CDER to send a letter to fluoridation manufacturers advising them to make FDA CDER NDA (New Drug Application) as required by Congress in the US FD&C Act.

a. In 1975, Drug Digest reported FDA CDER (Center for Drug Evaluation and Research) protected the public by withdrawing NDA (New Drug Application) for fluoride supplements (pills). FDA CDER must do the same for artificial fluoridation drug manufacturers. There is no difference in intent or efficacy between fluoride in pills and fluoridated water. But there is a significant difference in freedom of choice, labeling, and oversight.

b. **HHS** would incur no cost to request FDA CDER to take regulatory action.

c. **FDA CDER** would incur no cost to send a letter to artificial fluoridation drug manufacturers requiring them to gain NDA as required by law.

d. **FD&C Act** protects the public by requiring ***manufacturers*** to gain NDA, not the FDA nor patients. The FDA CDER is to evaluate and regulate substances used with the intent to prevent disease or listed in the official US Pharmacopoeia as a drug. Fluoride is used with intent to prevent disease and listed in the USP. The FDA has testified to Congress and the public that fluoride, when used with the intent to prevent disease, is a drug.

e. **CDC and Surgeon General** actively promote fluoridation for the manufacturers but do not determine scientifically the safety or efficacy of fluoridation or any drugs. Cities and water districts rely on the CDC and Surgeon General assuming they are correct.

f. **EPA** is prohibited by Congress from regulating the addition of any substance to water intended to treat humans. Fluoride is a protected pollutant and the EPA assumes efficacy.

g. **Excess exposure:** Of greatest concern is EPA's confirming in their Dose Response Analysis (DRA) that all infants on formula with fluoridated water are at risk. The DRA reports about a third of children under the age of 7 and all infants on formula made with fluoridated water will be ingesting too much fluoride under the proposed RfD (Reference Dose) and HHS proposed 0.7 ppm artificial fluoridation. Infants and children are being harmed. Excess exposure is confirmed with 41% of children now having dental fluorosis a biomarker of excess fluoride ingestion. An NDA would provide a legend, caution, warnings, and dosage, reducing risks.

h. Over 60 requests and petitions have been made to the FDA CDER since 2009 and the requests, petitions, and complaints have been made. These have been ignored, no answer, or pending for years.

2. **Instruct the CDC to stop the promotion (internet and education) of any and all drugs, including the ingestion of fluoride products, not FDA CDER approved.**

3. **Sponsor a review of the scientific evidence on fluoride's neurotoxicity by the National Academy of Science's National Research Council. The review should include studies listed at www.FluorideAlert.org/issues/health/brain**

Of most concern are the more than 30 human studies finding harm to brains. The question is no longer whether fluoridation causes neurological damage and lower IQ, the question is how much fluoride and at what age damage is caused.

Neurological harm is one of the reasons Israel recently banned fluoridation. Most developed countries have rejected fluoridation due to ethics, lack of efficacy and risks.

4. **Sponsor a quality published independent prospective randomized controlled trial (RCT), of the effectiveness of ingesting hydrofluorosilicic acid (fluoridation), including blood serum and urine concentrations of fluoride.**

a. Quality research is essential and in 60 years of fluoridation, not one published prospective randomized controlled trial of fluoridation has been done. Current reviews of the low quality research available are biased, serious unknowns are not controlled and even known confounding factors are often not controlled.

b. The results of a well-designed RCT could allow HHS to tailor public health policy on fluoridation to optimize benefits and minimize costs. This is in line with the goals of "Obamacare": evidence-based public health policy.

c. Most research on fluoridation have numerous problems which include:

- Not one Randomized Controlled Trial
- Socioeconomic status usually not controlled
- Inadequate size
- Difficulty in diagnosing decay
- Delay in tooth eruption
- Diet: Vitamin D, calcium, strontium, sugar, variables.
- Total exposure of Fluoride and measured blood and/or urine fluoride concentration
- Oral hygiene habits
- Not evaluating life time benefit
- Estimating or assuming subject actually drinks the fluoridated water.
- Dental treatment expenses
- Breast feeding and infant formula
- Fraud or gross errors.
- Genetics

Sincerely,

Jill McElheney
Chris Nidel JD
Bill Hirzy PhD
Paul Connett PhD
Bill Osmunson DDS, MPH

B62

Gerald Steel PE
Attorney at Law
7303 Young Rd. NW
Olympia WA 98502
360.867.1166 Phone

December 23, 2013

Ms. Jill Warner,
Acting Assoc. Commissioner
WO32, Room 5162
10903 New Hampshire Ave
Silver Spring, MD 20993

RE: Request for Review pursuant to 21 CFR 10.75 – Kailin System Public Drinking Water with Sodium Fluoride – Your file: RFD130073

Dear Ms. Warner:

On September 27, 2013, Leigh Hayes sent me the FDA determination (Attachments A-1 to A-3 hereto) wherein FDA states that it has determined that "Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the Federal Food, Drug, and Cosmetic Act (FD&C Act)." As a consequence, FDA has responded to our Request for Designation (RFD130073) by finding that our proposed fluoridated public drinking water is not a drug under the FD&C Act. On December 4, 2013, Leigh Hayes informed me that we can request review under 21 CFR 10.75. We hereby submit a Request for Review under 21 CFR 10.75 of the determinations regarding RFD130073.

The FDA has a long history of protecting the public from unsafe and ineffective drugs. Generally, state and local governments do not have the capability or staff to determine if articles or substances intended for preventative health care purposes are safe and effective. HHS, generally acting through the FDA, is the only regulatory body that has the authority to implement the FD&C Act in interstate commerce and protect the public from such articles and substances that are not safe and effective. So we ask the FDA to review its determination that our proposed "fluoridated public drinking water" is not a drug under the FD&C Act.

I believe that the FDA has accepted our statement of facts as accurate. Sodium Fluoride, as a water additive certified under industrial ANSI/NSF Standard 60 is intended for use in the prevention of tooth decay disease in man. (RFD130073 – our RFD at pages 1 and A-1.) This chemical with this intended use is square within the literal language included in the definition of a drug by Congress in 21 USC 321(g)(1)(B). (RFD130073 – our RFD at page 6.) When this chemical is added to our public drinking water, this chemical retains its intended use (prevention of tooth decay disease in man). The purpose of adding this chemical to our public drinking water is to deliver this chemical in drinking water for its intended use. As we stated, our "fluoridated public drinking water" is "intended for use in the prevention of dental caries (tooth decay) disease in man." (RFD130073 – our RFD at page 1.) With this statement, our "fluoridated public drinking water" is square within the literal language included in the definition of a drug in 21 USC 321(g)(1)(B).

RFD130073 provided a letter signed by EPA Water Law Office Associate General Counsel Steven M. Neugeboren, which was sent to me in 2013 on behalf of the EPA Administrator, and

 B63

which states the EPA official position that, "The Department of Health and Human Services (HHS) acting through the FDA, remains responsible for regulating the addition of drugs to water supplies for health care purposes." (RFD130073 – our RFD at page A-8 to A-9.) In RFD130073, we also cited to the Federal Supreme Court ruling in *United States v. An Article of Drug . . . Bacto-Unidisk* (*Bacto-Unidisk*), 394 U.S. 784, 793-801, 89 S.Ct. 1410, 22 L.Ed.2d 726 (1969) which found that the definition of "drug" in 21 USC 321(g)(1)(B) is "as broad as its literal language indicates." (RFD130073 – our RFD at page 6.) There can be no doubt that under the facts presented, ANSI/NSF Standard 60 certified Sodium Fluoride alone and our proposed fluoridated public drinking water are within the literal plain language of the definition of a drug in 21 USC 321(g)(1)(B). Therefore we continue to assert that such Sodium Fluoride and the proposed fluoridated public drinking water are drugs under federal law and are under the jurisdiction of FDA CDER.

I think we can assume that in 1974 Congress was aware of the definition of "drug" in 21 USC 321(g)(1)(B) and aware of the 1969 federal Supreme Court ruling in *Bacto-Unidisk*. I find no plain language in the 1974 SDWA (as amended) that seeks to carve out an exemption from the plain language of 21 USC 321(g)(1)(B) for fluoride water additives or fluoridated public drinking water when the intended use is for the prevention of dental caries disease in man. The challenged determination incorrectly claims that the "text" of the SDWA includes such [plain] language. It does not. The challenged determination also incorrectly claims support from the legislative history of the SDWA. The legislative history of the SDWA cannot be used by FDA to modify the plain language definition of "drug" in 21 USC 321(g)(1)(B) or modify the *Bacto-Unidisk* Court's interpretation of that drug definition. We request that you reverse the determination made for RFD130073 because the SDWA does not carve out an exemption from the plain language of 21 USC 321(g)(1)(B).

We claim that the intent of Congress is clear in 21 USC 321(g)(1)(B) as interpreted by *Bacto-Unidisk* that under our facts, ANSI/NSF Standard 60 certified Sodium Fluoride alone and our proposed fluoridated public drinking water are drugs under the FD&C Act. To further support our claim, we cited to 21 USC 321ff ("Dietary Supplement Health and Education Act of 1994") that states that minerals [such as fluoride public water additives] are foods except when they meet the definition of a drug. (RFD130073 – our RFD at page 6.) This 1994 statute did not exempt minerals that meet the definition of a "drug" in 21 USC 321(g)(1)(B) from being drugs just because the minerals were being added to public water supplies. This subsequent Congressional enactment supports our claim.

The federal Supreme Court in *FDA v. Brown & Williamson Tobacco Corp.* (*Tobacco Corp.*), 529 U.S. 120, 120 S.Ct. 1291, 146 L.Ed.2d 121 (2000) further supports our claim and refutes the claim in the determination regarding Congressional intent of 21 USC 321(g)(1)(B). The *Tobacco Corp.* Court found that reading the FD&C Act as a whole, as well as in conjunction with Congress' subsequent tobacco-specific legislation, it is plain that Congress has not given the FDA the authority to regulate tobacco products as customarily marketed. (*Tobacco Corp.* at 120 and 131-61.) "As customarily marketed" means "without manufacturer claims of therapeutic benefit." (*Id.* at 120.) But the *Tobacco Corp.* Court found that while the FDA did not generally have authority to regulate tobacco under the FD&C Act, there was a "well-established exception of when the manufacturer makes express claims of therapeutic benefit." (*Id.* at 158.) Therapeutic benefit refers to uses identified in 21 USC 321(g)(1)(B). We are making an express claim of therapeutic benefit for our proposed fluoridated public drinking water.

In the instant case, Congress has not shown that it has created a distinct regulatory scheme addressing the subject of purposely adding fluoride to public drinking water. But even if it did

B 64

have such a distinct regulatory scheme, FDA still has authority and responsibility under the FD&C Act to regulate fluoride added to public drinking water when it is added for the "therapeutic benefit" of preventing tooth decay disease. Similarly, FDA has authority and responsibility under the FD&C Act to regulate our fluoridated public drinking water because our water is fluoridated with the intent to prevent tooth decay disease. The FDA can point to no relevant federal caselaw where products that are intended for use in the prevention of disease in man are not regulated by the FD&C Act independent of other Congressional enactments.

Therefore under 21 CFR 10.75(a)(3) and 21 CFR 10.75(c)(1) and (2) along with 21 CFR 10.75(d) we request review and if it is concluded that our proposed ANSI/NSF Standard 60 fluoride water additives and our proposed fluoridated public drinking water are drugs, we again request that you designate our proposed fluoridated public drinking as a drug regulated by CDER.

Respectfully submitted,

Gerald Steel, PE, Attorney at Law
geraldsteel@yahoo.com

Attachments: A-1 to A-3

B 65

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Silver Spring, MD 20993

Office of Combination Products
WO 32, Room 5129
10903 New Hampshire Avenue
Silver Spring, MD 20993

September 27, 2013

Eloise Kailin
Owner and Manager
Gerald Steel
Attorney
Kailin Public Water System
160 Kane Lane
Sequim, WA 98382

Re: Request for Designation
Kailin Public Drinking Water System with Sodium Fluoride
Our file: RFD130073
Dated: July 22, 2013
Received: July 23, 2013
Filed: July 29, 2013

Dear Dr. Kailin and Mr. Steel:

The United States (U.S.) Food and Drug Administration (FDA) has completed its review of the request for designation (RFD) for the Kailin Public Drinking Water System with Sodium Fluoride that you submitted on behalf of Kailin Public Water System. We have determined that Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the Federal Food, Drug, and Cosmetic Act (FD&C Act). Instead, Congress intended that the U.S. Environmental Protection Agency (EPA) regulate fluoride in public drinking water as a potential contaminant under the Safe Drinking Water Act of 1974 (SDWA) to protect against adverse health effects, and that within the limits thus set by EPA, state and local governments be permitted, but not required, to fluoridate drinking water to help prevent dental caries. Thus, we are not designating your fluoridated public drinking water as a drug under the FD&C Act.

A-1 B 66

Description

In your RFD, you seek designation of your specific public fluoridated drinking water as a drug under the FD&C Act. You assert that you will submit a New Drug Application (NDA) for your fluoridated public drinking water that "will be composed of our public drinking water with an added fluoridation product certified to meet ANSI/NSF Standard 60...: Sodium Fluoride with a maximum addition of 2.3 mg/L....The public drinking water system is registered with the Washington State Department of Health as PWS ID# AC982. It is a neighborhood system with multiple approved connections. The source water comes from a well as is typical for public water systems in Washington State and currently there is a transmission pipeline from the well to a tank that maintains water pressure for the system in an acceptable range. A distribution system which starts at the tank serves all of the individual residential and commercial connections. There are pressure zones in the distribution system where pressure reducers are used to lower water pressure for connections at lower elevations. All individual connections to the distribution system are made in a manner approved by the Washington State Department of Health."

The RFD explains that "...the transmission line will be rerouted to a small fluoridation building where fluoridation will occur and the fluoridated water will be transmitted to the tank that maintains water pressure. This public water system is required to meet standard specifications for public water systems in Washington State as established by the Washington State Board of Health." The RFD states that the addition of the fluoridation materials "...will be metered into flowing water in a manner to maintain the specified chemical concentration rates. The Sodium Fluoride will be injected using an up-draft fluoride saturator. The injection rate into the transmission line in the control house will be controlled using a 4 to 20 milliamperes signal from the main water meter so that finished fluoridation levels are close to 0.7 mg/L. Fluoride levels will be manually checked twice daily." Finally, with regard to packaging of the product, the RFD asserts that "[t]his system does not have conventional packaging. [The company proposes] that [it] will negotiate with CDER regarding adequate labeling. For example, [the company] will propose that drug facts and warning approved by CDER will be sent out with each billing for each connection."

You recommend that your fluoridated public drinking water designed to aid in the prevention and prophylactic treatment of dental caries disease be classified as a drug and that it be assigned to FDA's Center for Drug Evaluation and Research (CDER) for premarket review and regulation.

Product Classification

We have considered the information in the RFD and discussed the issues with staff from CDER, the Center for Food Safety and Applied Nutrition, the Department of Health and Human Services, HHS's Office of the General Counsel, and the EPA.

A-2 B67

After careful consideration, we conclude that Congress did not intend for FDA to regulate the addition of fluoride to public drinking water for dental caries prevention as a drug under the FD&C Act. Instead, Congress intended that EPA regulate fluoride in public drinking water as a potential contaminant under the SDWA to protect against adverse health effects, and that within the limits thus set by EPA, state and local governments be permitted, but not required, to fluoridate public drinking water to help prevent dental caries. The SDWA gives EPA certain authorities with respect to the regulation of public drinking water, including the authority to promulgate national primary drinking water regulations that set maximum contaminant levels (MCLs) for contaminants that EPA determines may have an adverse effect on human health. Pursuant to its authority under the SDWA, EPA has codified a primary MCL for fluoride at 40 CFR § 141.62(b)(1) and a secondary MCL for fluoride at 40 CFR § 143.3.

The historical context surrounding the passage of the SDWA indicates that Congress was aware in 1974 that many localities were adding fluoride to public drinking water to help prevent dental caries. They were also aware that FDA had a codified policy of not regulating such fluoride as a drug, so long as the levels were within certain recommended limits. Based on the text and legislative history of the SDWA, we have concluded that Congress did not intend for FDA to regulate fluoride in public drinking water for the purpose of helping to prevent dental caries as a drug under the FD&C Act. Instead, Congress set up a regime under which EPA would set upper limits for fluoride to protect against adverse health effects, and EPA would not have the authority to mandate or ban the use of fluoride to help prevent dental caries. The decision of whether or not to add fluoride to public drinking water to help prevent dental caries (within the limits set by EPA) was left to state and local authorities, as it had been before 1974. Since the passage of the SDWA, this division of federal and state/local oversight has continued.

Conclusion

For the reasons explained above, we have determined that Congress did not intend for FDA to regulate fluoride in public drinking water to help prevent dental caries as a drug under the FD&C Act, and we therefore are not designating your fluoridated public drinking water as a drug.

If you have any other questions about this letter, please feel free to contact me. You may reach us at the above address or by email at combination@fda.gov.

Sincerely,

Leigh Hayes

Leigh Hayes
Product Assignment Officer

B 68

A-3

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
GENERAL COUNSEL

February 14, 2013

Gerald Steel, PE
7303 Young Road NW
Olympia, WA 98502

Dear Mr. Steel:

This is in response to your letter of December 28, 2012 to EPA Administrator Lisa Jackson in which you asked several questions about the status of an MOU between EPA and the Federal Drug Administration (FDA) published in 1979. I am replying on behalf of her.

Your first question is whether, from the viewpoint of EPA, the purpose of a 1979 Memorandum of Understanding (MOU) between EPA and the Federal Drug Administration (FDA) was "to take away from FDA, and give to EPA, responsibility for regulating public drinking water additives intended for preventative health care purposes and unrelated to contamination of public drinking water?" Your second question is whether, if that was the purpose of the 1979 MOU, the MOU was terminated through a subsequent Federal Register notice.

The answer to your first question is no, so there is no need to address your second question. The purpose of the MOU was not to shift any responsibilities between the Agencies. Rather, it was to help facilitate effective coordination of our respective legal authorities. Under the Safe Drinking Water Act (SDWA), EPA is the lead federal agency with responsibility to regulate the safety of public water supplies. EPA does not have responsibility for substances added to water solely for preventative health care purposes, such as fluoride, other than to limit the addition of such substances to protect public health or to prevent such substances from interfering with the effectiveness of any required treatment techniques. SDWA Section 1412(b)(11); see also A Legislative History of the Safe Drinking Water Act, Committee Print, 97th Cong, 2d Session (February 1982) at 547. The Department of Health and Human Services (HHS), acting through the FDA, remains responsible for regulating the addition of drugs to water supplies for health care purposes.

The 1979 MOU was intended to address contamination of drinking water supplies as a result of direct or indirect additives to drinking water, not to address the addition of substances solely for preventative health purposes. 44 Fed. Reg. 42775 (July 20, 1979) ("EPA and FDA agree: (1) that *contamination* of drinking water from the use and application of direct and indirect additives and other substances poses a potential public health problem...")(emphasis added). It was intended to avoid potentially duplicative regulation of "food", which FDA had, in the past, considered to include drinking water. 44 Fed. Reg. 42775 (July 20, 1979). The MOU did not address drugs or other substances added to water for health care purposes.

B69

I hope that this has adequately answered your inquiry. Please do not hesitate to contact Carrie Wehling of my staff (202-564-5492) if you have further questions about this.

Sincerely,

Steven M. Neugeboren
Associate General Counsel
Water Law Office

B 70

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 10
1200 Sixth Avenue, Suite 900
Seattle, WA 98101-3140

OFFICE OF
WATER AND
WATERSHEDS

NOV 17 2011

Mr. Gerald Steel, PE
Attorney-at-Law
7303 Young Road NW
Olympia, Washington 98502

Dear Mr. Steel:

I am responding to your letter dated November 7, 2011, on behalf of Dennis J. McLerran, Regional Administrator, U S. Environmental Protection Agency (EPA). In your communication you have asked the EPA to send you a letter that answers the question "Are [Washington Administrative Code] WAC 246-290-220(3) and 246-290-460 part of implementation of requirements of the Federal Safe Drinking Water Act in Washington State, or are they unrelated to the requirements of the Federal Safe Drinking Water Act in Washington State?"

A concise answer to your question is that the provisions at WAC 246-290-220(3) and 246-290-460 are not related to the requirements of the Federal Safe Drinking Water Act in Washington State. An explanation as to why this is the case follows.

The requirements for a State drinking water primacy program are spelled out in Section 1413 of the Federal Safe Drinking Water Act (SDWA) (42 U.S.C. § 300g-2). Section 1413(a) specifies that a State has primary enforcement responsibility (primacy) for public water systems during any period for which the EPA Administrator determines that such State:

(1) has adopted drinking water regulations that are no less stringent than the national primary drinking water regulations i.e., the regulations promulgated at 40 CFR Part 141 (see http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?tpl=/ecfrbrowse/Title40/40tab_02.tpl);

(2) has adopted and is implementing adequate procedures for the enforcement of such State regulations as the Administrator may require by regulation;

(3) will keep such records and make such reports with respect to its activities as the Administrator may require by regulation;

(4) if it permits variances and/or exemptions from the requirements of its drinking water regulations, permits such variances and exemptions under conditions and in a manner which is not less stringent than the conditions under, and the manner in which variances and exemptions may be granted under SDWA sections 1415 and 1416;

(5) has adopted and can implement an adequate plan for the provision of safe drinking water under emergency circumstances; and

(6) has adopted authority for administrative penalties, unless the constitution of the State prohibits the adoption of such authority.

B 71

The EPA's role in SDWA section 1413(b) requires the Administrator to promulgate regulations that establish how the States may apply for primacy, how the Administrator will make primacy determinations and the manner in which the Administrator may determine that the primacy agency is no longer meeting the primacy requirements. These primacy implementing regulations can be found at 40 CFR 142.10 – 40 CFR 142.12. (See enclosure and/or website provided above.) 40 CFR Part 142.10 describes the requirements of a State primacy program. 40 CFR Part 142.11 describes the documents a State must submit to the EPA for an initial determination of primacy. 40 CFR 142.12 describes the contents of a State request for approval of a State's revised primacy program. This must take place whenever the EPA adopts a new or revised drinking water rule. As per 40 CFR 142.12(c) a State must submit for EPA approval a copy of their regulations and a document we refer to as a crosswalk. The crosswalk is a side-by-side comparison of the new or revised Federal requirements in 40 CFR Parts 141 and 142 and the corresponding State authorities, including citations to the specific statutes and administrative regulations (see enclosed example of a crosswalk page). EPA will only make a determination that a State's revised drinking water primacy program can be approved if the State's regulations are as stringent as the Federal regulations and the State continues to maintain all required authorities as per SDWA Section 1413.

WAC 246-290-220(3) requires treatment chemicals with the exception of commercially retailed hypochlorite compounds added to water intended for potable use to comply with ANSI/NSF Standard 60 and also specifies that the maximum application dosage recommendation for the product certified by the ANSI/NSF Standard 60 shall not be exceeded in practice. The Department of Health (DOH), which is the State of Washington's drinking water primacy agency has never submitted WAC 246-290-220(3) to the EPA for approval as there is no analogous provision in the National Primary Drinking Water Regulations at 40 CFR Part 141, and neither the other statutory provisions mentioned above, nor the primacy implementing provisions at 40 CFR Part 142 require that language, such as is found in WAC 246-290-220(3), be part of a State primacy program.

WAC 246-290-460 addresses fluoridation practices, should a community choose to provide fluoridation. DOH has never submitted WAC 246-290-460 to the EPA for approval as there are no analogous provisions in the National Primary Drinking Water Regulations at 40 CFR Part 141, and neither the other statutory provisions mentioned above, nor the primacy implementing provisions at 40 CFR Part 142 require that a State primacy program regulate fluoridation practices.

For the reasons stated in the above paragraphs, I can assert that that the provisions at WAC 246-290-220(3) and 246-290-460 were not required to be submitted by the State or approved by the EPA and these provisions are not related to the requirements of the Federal Safe Drinking Water Act.

I hope this response answers your questions satisfactorily. If you have additional questions, please contact Marie Jennings, our Manager for the Drinking Water Unit at (260) 553-1893.

Sincerely,

Michael A. Bussell, Director
Office of Water & Watersheds

Enclosures

B 72

February 2008

NSF Fact Sheet on Fluoridation Chemicals

Introduction

This fact sheet provides information on the fluoride containing water treatment additives that NSF has tested and certified to NSF/ANSI Standard 60: Drinking Water Chemicals - Health Effects. According to the latest Association of State Drinking Water Administrators Survey on State Adoption of NSF/ANSI Standards 60 and 61, 45 states require that chemicals used in treating potable water must meet Standard 60 requirements. If you have questions on your state's requirements, or how the NSF/ANSI Standard 60 certified products are used in your state, you should contact your state's Drinking Water Administrator.

Water fluoridation is the practice of adjusting the fluoride content of drinking water. Fluoride is added to water for the public health benefit of preventing and reducing tooth decay and improving the health of the community. The U.S. Centers for Disease Control and Prevention is a reliable source of information on this important public health intervention. For more information please visit www.cdc.gov/fluoridation/.

NSF certifies three basic products in the fluoridation category:

1. Fluorosilicic Acid (aka Fluosilicic Acid or Hydrofluosilicic Acid).
2. Sodium Fluorosilicate (aka Sodium Silicofluoride).
3. Sodium Fluoride.

NSF Standard 60

Products used for drinking water treatment are evaluated to the criteria specified in NSF/ANSI Standard 60. This standard was developed by an NSF-led consortium, including the American Water Works Association (AWWA), the American Water Works Association Research Foundation (AWWARF), the Association of State Drinking Water Administrators (ASDWA), and the Conference of State Health and Environmental Managers (COSHEM). This group developed NSF/ANSI Standard 60, at the request of the US EPA Office of Water, in 1988. The NSF Joint Committee on Drinking Water Additives continues to review and maintain the standard annually. This committee consists of representatives from the original stakeholder groups as well as other regulatory, water utility and product manufacturer representatives.

Standard 60 was developed to establish minimum requirements for the control of potential adverse human health effects from products added directly to water during its treatment, storage and distribution. The standard requires a full formulation disclosure of each chemical ingredient in a product. It also requires a toxicology review to determine that the product is safe at its maximum use level and to evaluate potential contaminants in the product. The standard requires testing of the treatment chemical products, typically by dosing these in water at 10 times the maximum use level, so that trace levels of contaminants can be detected. A toxicology evaluation of test results is required to determine if any contaminant concentrations have the potential to cause adverse human health effects. The standard sets criteria for the establishment of single product allowable concentrations (SPAC) of each respective contaminant. For contaminants regulated by the U.S. EPA, this SPAC has a default level not to exceed ten-percent of the regulatory level to provide protection for the consumer in the unlikely event of multiple sources of the contaminant, unless a lower or higher number of sources can be specifically identified.

B 73

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Silver Spring, MD 20993

Office of Combination Products
WO 32, Room 5129
10903 New Hampshire Avenue
Silver Spring, MD 20993

September 23, 2015

Mr. Gerald Steel, PE
Attorney-At-Law
7303 Young Road, NW
Olympia, WA 98502

Re: "Submittal of Three Requests for Designation
for Libera Bottled Fluoridated Water each using a Different Fluoridation Chemical"
Dated: September 2, 2015
Received: September 2, 2015

Dear Mr. Steel:

For the reasons discussed below, we disagree that our previous legal reasoning is, as you indicate below, "no longer valid." As we have previously communicated to you, and as stated in the preamble to 21 CFR Part 3, Part 3 "does not apply to foods, veterinary products, or cosmetics" (56 FR 58754), and jurisdictional questions concerning a product that may be within the jurisdiction of the Center for Food Safety and Applied Nutrition (CFSAN) are outside the scope of 21 CFR part 3 and section 563 of the FD&C Act. In contrast to your characterization, the Center for Food Safety and Applied Nutrition's (CFSAN's) recent communication to you (Letter from F. Billingslea dated August 7, 2015, attached) does not state that your proposed bottled water product with the claim discussed below ("this drinking water is intended for use in the prevention of tooth decay disease") is "not a food under their [CFSAN's] jurisdiction." Instead, Ms. Billingslea stated that this proposed labeling statement "is not an authorized claim on food labeling under Section 403(r) of the Act." Ms. Billingslea further recommended that you contact Ms. Barbara Gould in FDA's Center for Drug Evaluation and Research (CDER) if you wished to market a bottled water product with this claim.

Ms. Billingslea recommended contacting CDER because you propose to market your product with a therapeutic claim. Your proposed claim would establish that your product is intended to prevent disease. Therefore, your proposed product (if marketed with your proposed claim) would be a drug as that term is defined in section 201(g)(1)(B) of the Federal Food, Drug, and Cosmetic Act (the Act).

B74

However, the fact that your proposed product (if marketed with your proposed claim) would be a drug under the Act does not mean that your product is not also a food. To the contrary, the definitions of "food" and "drug" under the Act are not mutually exclusive. *See, e.g., Nutrilab v. Schweiker*, 713 F.2d 335, 336 (7th Cir. 1983). It is commonplace for FDA to take regulatory action with respect to food products that are promoted for conditions that cause the products to be drugs as well as foods.

Accordingly, we believe that our previous legal reasoning continues to apply, and your most recent requests fall outside the scope of the regulation and statutory provisions that authorize requests for designation. As a result, we are not treating your submissions regarding fluoridated bottled water as requests for designation. Instead, we are treating them as informal inquiries.

We hope it is helpful for you to know that your proposed product (if marketed with your proposed claim) would be both a food and a drug under the Act. We note that if you were to remove your proposed claim ("This drinking water is intended for use in the prevention of tooth decay disease"), your product would not be a drug under the Act unless there was other evidence to establish its status as a drug. As Ms. Billingslea discussed in her letter of August 7, your other proposed claim – "fluoride added" – would not render your product a drug. You can also reference Ms. Billingslea's letter for information about a health claim that may be used on certain bottled water products.

As Ms. Billingslea stated in her letter of August 7, we recommend that you contact Ms. Barbara Gould in CDER if you wish to market your bottled water product with your proposed claim about the prevention of tooth decay.

Patricia A. Hansen, Ph.D.

Acting Director
Office of Nutrition, Labeling and Dietary Supplements
CFSAN
FDA

Leigh Hayes

Leigh Hayes

Product Assignment Officer
Office of Combination Products
FDA

B 75

CFSAN/Office of Nutritional Products, Labeling, and Dietary Supplements
October 14, 2006

Health Claim Notification for Fluoridated Water and Reduced Risk of Dental Caries

Under section 403(r)(3)(C) (21 U.S.C. §343(r)(3)(C)) of the Federal Food, Drug, and Cosmetic Act (Act), a manufacturer may submit to the Food and Drug Administration (FDA) a notification of a health claim based on an authoritative statement from an appropriate scientific body of the United States Government or the National Academy of Sciences (NAS) or any of its subdivisions. The notification must be submitted to FDA at least 120 days before the food is introduced into interstate commerce. The claim may be made after 120 days from the date of submission of the notification until such time as 1) FDA issues a regulation prohibiting or modifying the claim or finding that the requirements for making the claim have not been met, or 2) a district court in an enforcement proceeding has determined that the requirements for making the claim have not been met.

On June 16, 2006, the FDA received a notification (the June 16 notification) from the law firm of Covington and Burling regarding a health claim for the relationship between fluoridated water and a reduced risk of dental caries. The 120-day period from the date of submission of the June 16 notification was October 14, 2006. Therefore, after October 14, 2006, manufacturers may use the claim specified in the notification, as modified by the notifier in a letter to FDA dated October 13, on the label and in labeling of any food product that meets the eligibility criteria described below, unless or until FDA or a court acts to prohibit the claim.

The June 16 notification cites statements from several sources as authoritative statements for the claim. FDA reviewed the sources and cited statements in their context and in light of existing authorized health claims and current science. The following three statements are considered authoritative for purposes of this notification.

Recommendation for Using Fluoride to Prevent and Control Dental Caries in the U.S. (Centers for Disease Control, 2001):

> "Widespread use of fluoride has been a major factor in the decline in the prevalence and severity of dental caries (i.e., tooth decay) in the United States and other economically developed countries. When used appropriately, fluoride is both safe and effective in preventing and controlling dental caries. All U.S. residents are likely exposed to some degree of fluoride, which is available from multiple sources." (Summary section, page 1)
>
> "Continue and extend fluoridation of community drinking water: Community water fluoridation is a safe, effective, and inexpensive way to prevent dental caries. This modality benefits persons in all age groups and of all SES, ..." (Recommendation section, page 24)

Oral Health in America: A Report of the Surgeon General (2000):

> "Community water fluoridation is safe and effective in preventing dental caries in both children and adults. Water fluoridation benefits all residents served by community water supplies regardless of their social or economic status. Professional and individual measures, including the use of fluoride mouth rinses, gels, dentifrices, and dietary supplements and the application of dental sealants, are additional means of preventing dental caries." (Executive summary)

Review of Fluoride: Benefits and Risks (Public Health Service, 1991):

> "Extensive studies over the past 50 years have established that individuals whose drinking water is fluoridated show a reduction in dental caries. Although the comparative degree of measurable benefit has been reduced recently as other fluoride sources have become available in non-fluoride areas, the benefits of water fluoridation are still clearly evident." (Conclusions section, page 87)

According to the June 16 notification and the letter to FDA dated October 13, the food eligible to bear the claim is bottled water meeting the standards of identity and quality set forth in 21 CFR 165.110, containing greater than 0.6 and up to 1.0 mg/L *total* fluoride, and meeting all general requirements for health claims (21 CFR 101.14) with the exception of minimum nutrient contribution (21 CFR 101.14 (e)(6)). The claim language is: "Drinking fluoridated water may reduce the risk of [dental caries or tooth decay]." In addition, the health claim is not intended for use on bottled water products specifically marketed for use by infants.

The notification and materials regarding the claim are publicly available from the FDA Division of Dockets Management (Docket No.2006Q-0418). Persons interested in these documents may view them at the Division of Dockets Management from 9am to 4pm, Monday through Friday at 5630 Fishers Lane, room 1061, Rockville, MD 20852. The Division of Dockets Management may be contacted at 301-827-6860. FDA also intends to make the documents available on the Dockets web site at http://www.fda.gov/ohrms/dockets/dockets/dockets.htm, under Docket No. 2006Q-0418.

FDA U.S. FOOD & DRUG ADMINISTRATION

Inspections, Compliance, Enforcement, and Criminal Investigations

DS Waters of America, LP 6/8/09

Department of Health and Human Services

Public Health Service
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

JUN - 8 2009

WARNING LETTER

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Mr. Stewart E. Allen
Mr. Dillon Schickli
Chief Executive Officers
DS Waters of America, Inc.
5660 New Northside Drive, Suite 500
Atlanta, GA 30328

Re: CFSAN-OC-WL09-03

Dear Mr. Allen and Schickli:

The Food and Drug Administration (FDA) has reviewed the label for the 1 Gal (3.78L) package of your NURSERY@ Purified Water with added fluoride, and your website at www.nurserywater.com in April 2009. Based on our review, we have found that your product label has serious violations of the Federal Food, Drug, and Cosmetic Act (the Act) and the applicable regulations in Title 21, Code of Federal Regulations (21 CFR). You can find the Act and these regulations through links on FDA's Internet website at www.fda.gov.

Your significant violations are as follows:

Your product is misbranded within the meaning of section 403(r)(1)(B) of the Act [21 U.S.C. 343(r)(1)(B)], because it bears an unauthorized health claim in its labeling. We have determined that your website www.nurserywater.com. the address for which appears on your product's label, is labeling for your product under section 201(m) of the Act [21 U.S.C. 321(m)]. This website bears the following unauthorized health claim: "Drinking water with added fluoride in the proper amounts has been shown to be so effective that the ADA supports municipal water fluoridation and refers to this as the single most effective measure to prevent tooth decay." Health claims may not be made for food products, including bottled water, for which the label represents or purports that the food is for infants or toddlers less than two years of age, unless FDA has provided for such claim by regulation. 21 CFR 101.14(e)(5). [1]

Your product is misbranded within the meaning of section 403(q) of the Act [21 U.S.C. 343(q)] in that nutrition facts information is not in an appropriate format as defined in 21 CFR 101.9. The Nutrition Facts panel uses abbreviations for serving size and servings per container that are not in accordance with 21 CFR 101.9(j)(13)(ii)(B), which provides that the use of specific abbreviations to list nutrients is only for packages that have a total surface area available to bear labeling of 40 or less square inches. In addition, the correct heading on the Nutrition Facts panel for declaring the quantity of a nutrient is "% Daily Value" (not "Amount") in accordance with 21 CFR 101.9(d)(6).

This letter is not meant to be an all-inclusive review of your NURSERY® Purified Water product and its labeling. It is your

responsibility to ensure that all of your products are in compliance with the laws and regulations enforced by FDA. You should take prompt action to correct the violations described above and prevent their recurrence. Failure to promptly correct these violations may result in regulatory action without further notice, such as seizure and/or injunction.

Please respond in writing within fifteen (15) working days from your receipt of this letter. Your response should include each step that has been or will be taken to completely correct the labeling violations and to prevent the recurrence of similar violations, the time within which the correction will be completed, and any documentation necessary to show that the correction has been achieved. If applicable, please include a copy of your revised label. If corrective actions cannot be completed within fifteen (15) working days, state the reason for the delay and the time within which the corrections will be completed.

In addition, we have the following comments:

- The serving size of your NURSERY® Purified Water product is based on 8 fluid ounces. While FDA has not established a reference amount customarily consumed (RACC) for water by infants and toddlers, we recommend that you use the infant and toddler RACC for juices, which is 4 fl oz (120 mL) [21 CFR 101.12(b), Table I (Juices, all varieties)].
- Although minerals are added for taste, the statement of identity does not include this information (e.g., "purified water with minerals added for taste") [60 FR 57076 at 57080 and 57082, November 13, 1995].

If you need additional information or have questions concerning any products distributed through your website, please contact FDA. You may respond in writing to Felicia Binion Williams, Compliance Officer, Division of Enforcement, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740.

Sincerely,

/s/

Roberta F. Wagner

Director
Office of Compliance
Center for Food Safety
and Applied Nutrition

(b)(4)

1. FDA notes that the following claim regarding fluoridated water and reduced risk of dental caries or tooth decay may be made consistent with a health claim notification under section 403(r)(3)(C) of the Act [21 U.S.C. 343(r)(3)(C)]: "Drinking fluoridated water may reduce the risk of [dental caries or tooth decay]." [www.cfsan.fda.gov/~dms/flfluoro.html]. However, the prohibition under 21 CFR 101.14(e)(5) applies to these health claim notifications, and this notification explicitly excluded bottled water products that are specifically marketed for use by infants, and therefore your product is not eligible to bear this health claim. Furthermore, the language of the claim on your website differs significantly from the language in the claim in the notification.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

FEB 21 1997

John V. Kelly
Assemblyman, 36th District
Bergen-Essex-Passaic Countries
371 Franklin Avenue, 2nd Floor
Nutely, New Jersey 07110

Dear Assemblyman Kelly:

This responds to your February 5, 1997, letter asking whether there have been any drug applications approved by the Food and Drug Administration for fluoride tablets for children or fluoride drops for infants since 1992.

At this time there are no approved new drug applications on file with the Agency for these products. If you have any further questions on this issue I can be contacted at (301) 594-0101.

Sincerely yours,

Frank R. Fazzari

Frank R. Fazzari
Prescription Drug Strategy Development & PDMA
Division of Prescription Drug Compliance and Surveillance
Office of Compliance
Center for Drug Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

DEC 21 2000

The Honorable Ken Calvert
Chairman
Subcommittee on Energy and Environment
Committee on Science
House of Representatives
Washington, D.C. 20515-6301

Dear Mr. Chairman:

Thank you for the letter of May 8, 2000, to Dr. Jane E. Henney, Commissioner of Food and Drugs, regarding the use of fluoride in drinking water and drug products. We apologize for the delay in responding to you.

We have restated each of your questions, followed by our response.

1. **If health claims are made for fluoride-containing products (e.g. that they reduce dental caries incidence or reduce pathology from osteoporosis), do such claims mandate that the fluoride-containing product be considered a drug, and thus subject the product to applicable regulatory controls?**

Fluoride, when used in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or animal, is a drug that is subject to Food and Drug Administration (FDA) regulation. FDA published a final rule on October 6, 1995, for anticaries drug products for over-the-counter (OTC) human use (copy enclosed). This rule establishes the conditions under which OTC anticaries drug products are generally recognized as safe and effective and not misbranded. The rule has provisions for active ingredients, packaging conditions, labeling, and testing procedures that are required by manufacturers in order to market anticaries products. A new drug application (NDA) may be filed for a product containing fluoride that does not meet the provisions stated in the final rule. As you know, the Environmental Protection Agency regulates fluoride in the water supply.

2. Are there any New Drug Applications (NDA) on file, that have been approved, or that have been rejected, that involve a fluoride-containing product (including fluoride-containing vitamin products) intended for ingestion with the stated aim of reducing dental caries? If any such NDA's have been rejected, on what grounds were they rejected? If any such NDA have been approved, please provide the data on safety and efficacy that FDA found persuasive.

No NDAs have been approved or rejected for fluoride drugs meant for ingestion. Several NDAs have been approved for fluoride topical products such as dentifrices and gels. Fluoride products in the form of liquid and tablets meant for ingestion were in use prior to enactment of the Kefauver-Harris Amendments (Drug Amendments of 1962) to the Food, Drug, and Cosmetic Act in which efficacy became a requirement, in addition to safety, for drugs marketed in the United States (U.S.). Drugs in use prior to 1962 are being reviewed under a process known as the drug efficacy study implementation (DESI). The DESI review of fluoride-containing products has not been completed.

3. Does FDA consider dental fluorosis a sign of over exposure to fluoride?

Dental fluorosis is indicative of greater than optimal ingestion of fluoride. In 1988, the U.S. Surgeon General reported that dental fluorosis, while not a desirable condition, should be considered a cosmetic effect rather than an adverse health effect. Surgeon General M. Joycelyn Elders reaffirmed this position in 1994.

4. Does FDA have any action-level or other regulatory restriction or policy statement on fluoride exposure aimed at minimizing chronic toxicity in adults or children?

The monograph for OTC anticaries drug products sets acceptable concentrations for fluoride dentifrices, gels and rinses (all for topical use only). This monograph also describes the acceptable dosing regimens and labeling including warnings and directions for use. FDA's principal safety concern regarding fluoride in OTC drugs is the incidence of fluorosis in

children. Children under two years of age do not have control of their swallowing reflex and do not have the skills to expectorate toothpaste properly. Young children are most susceptible to mild fluorosis as a result of improper use and swallowing of a fluoride toothpaste. These concerns are addressed in the monograph by mandating maximum concentrations, labeling that specifies directions for use and age restrictions, and package size limits.

Thanks again for contacting us concerning this matter. If you have further questions, please let us know.

Sincerely,

Melinda K. Plaisier
Associate Commissioner
for Legislation

Enclosure
"Final Rule/Federal Register - October 6, 1995
Over-the-Counter Anticaries Drug Products"

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Bill Osmunson DDS , MPH
bill@teachingsmiles.com 425.466.0100
2011 Not updated

WASHINGTON STATE BOARD OF HEALTH

RE: FLUORIDE'S EFFECTS ON THE ENDOCRINE SYSTEM, January 2024

OUTLINE:

SUMMARY: Fluoride is an endocrine disruptor.

Maximum fluoride intake goal <0.001 mg/kg/day.

I. EVALUATING FLUORIDE AS AN ENDOCRINE DISRUPTING TOXICANT.

Hundreds of research articles have reported adverse effects of excess fluoride exposure including but not limited to arthritis, bone, tooth, brain, cancer, cardiovascular, diabetes, thyroid, parathyroid, pancreas, pineal, adrenal, gonads, enteroendocrine, paraganglia, pituitary, placenta, endocrine, GI, kidney, and reproductive harm.

Fluoride has effects throughout the body. Fluoride should be evaluated at the biochemical, cellular, and organ levels as well as synergistic toxic effects with a margin of safety for race, age, nutritional deficiencies, ill health of those most vulnerable, total exposure and unknowns. To protect the public, we must use a margin of safety from the lowest observed adverse effect and a factor of 100. We do a disservice to humanity and science when we compartmentalize evidence without bringing the weight from all effects to the table for evaluation and judgment. In the end, judgment is required from a "global" perspective for all, not just the mean.

The NRC (2006) report to the EPA which labeled fluoride an "endocrine disruptor,"[1] as well as numerous studies,[2] reviews, and reasonable judgment.

The NRC (2006)[3] review members were tasked to determine "with absolute certainty" that research had demonstrated adverse effects---one member remembers the term, "bet the farm certainty". Such a high degree of certainty is not supported by Congress who requires the EPA to determine contaminate levels to be *"set at the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety."* The committee unanimously "bet the farm" that fluoride is an endocrine disruptor.

The endocrine system includes all of the glands of the body and the hormones produced by those glands, such as anterior and posterior pituitary, thyroid, parathyroid, adrenal, gonads, islets of pancreas, pineal, enteroendocrine, paraganglia and placenta. The glands are controlled directly by stimulation from the nervous system as well as by chemical receptors in the blood and hormones produced by interaction with other glands. By regulating the functions of organs in the body, these glands help to maintain

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[1] National Academies of Sciences, Engineering and Medicine 500 Fifth St. N.w. Washington, DC, 20001. Page 266 "Fluoride in Drinking Water: A Scientific Review of EPA's Standards."

[2] Such as Malin A, Till C, Exposure to fluoridated water and attention deficit hyperactivity disorder prevalence among children and adolescents in the United States: an ecological association. Environmental Health (2015) 14:17 and
Peckham et al, (2015) Centre for Health Services Studies, University of Kent, Canterbury, Kent, UK. J. Epidemiology Community Health do:10.1136/jech-2014-204971

[3] "Fluoride in Drinking Water: A Scientific Review of EPA's Standards."
http://www.nap.edu/catalog/11571/fluoride-in-drinking-water-a-scientific-review-of-epas-standards

the body's homeostasis, such as cellular metabolism, reproduction, sexual development, sugar and mineral homeostasis, heart rate, and digestion. Research has only begun to glimpse into fluoride's effects on these systems; however, we have enough evidence to confidently state fluoride is an endocrine toxicant, a disruptor. Current research supports the NRC (2006) conclusion and provides greater evidence to establish a least observable effect with margin of safety. The question is no longer whether fluoride is safe, the question is "like lead, is any dosage of fluoride is safe for everyone?"

The endocrine system is closely connected to the neurological system such as through neurosecretors which release neurotransmitters into the blood through extracellular fluids. We may consider three major classes of molecules that function as hormones in vertebrates: 1. water soluble peptide hormones such as epinephrine, 2. lipid soluble/fluid hormones with receptor on the nucleus of target cells which turns on transcription quickly such as testosterone, 3. local regulators/paracrine signaling which convey messages between neighboring cells such as cytokines (immune response). Numerous hormones such as ADH, FSH, LH, ACTH, growth hormones, pituitary hormones, pancreatic hormones, insulin IGF, hypo- and hyperthyroidism, insulin (diabetes), glucagon, adrenal glands, need to be considered individually, synergistically, and as they effect the entire human body. We must not leave the public at risk, waiting for the patients (public) to provide absolute proof of harm, such as prospective randomized controlled trials of lower IQ, before governments stop mass medication of fluoride without consent for a nonlethal and noncontagious disease prevented with good hygiene and diet.

We have a null probability of fluoride being safe for everyone at EPA's MCL, especially when in combination with synergistic toxicants, compromised endocrine systems, or various ages and stages of life and at concentrations greater than mother's milk which in most samples has no detectible fluoride (mean 0.004 ppm or about 0.001 mg/Kg/day) and the longest running fluoride research project known. Until we have robust research proving the level of fluoride in mother's milk is deficient, incomplete, or defective; mother's milk should be the normative model against which all other infant formulas should be compared, **<0.001 mg/Kg/day.** Most infants (80%-90%) receive some or all formula usually reconstituted with public water resulting in about 175 to 250 times more fluoride than mother's milk, mean of 0.004 ppm. (most samples not detectible)

Therefore, the evidence of mother's milk may not fit into a formula, rubric or matrix but the weight of evidence should be used for common sense judgment. Judgment, keeping in mind the insufficient evidence of benefit, lack of individual informed consent and weight of all evidence of risks for each individual, not just the mean or 90th

percentile. Fluoride is an endocrine disruptor and should be treated as a toxicant like lead.

Consider Nakamoto (2018)[4] "Fluoride Exposure in Early Life as the Possible Root Cause of Disease In Later Life."

Mechanism of action

Fluorine enters the body by ingestion, respiration and skin absorption. Exposed tissues are utilized by HF in neutralization reactions leaving the fluoride ion free to pass further into the body. The fluoride anion reacts with HCl in the stomach to form HF. HF is then absorbed by the GI tract and passes into the liver via the portal vein. Elemental F is one of the strongest oxidizers currently known. The anion is immune to the body's first line of defense of biotransformation, phase 1 metabolic reactions, which are generally oxidative reactions in the liver. HF passes into the blood stream and to all tissues. Calcium in all tissues reacts with HF to form an insoluble salt, calcium fluoride. Calcium fluoride is cleared by the body, leaching out some calcium which would be part of the bones, teeth, pineal gland, nerves, etc. The process results in increased density and brittleness, compressive strength of bones and teeth, with decreased tinsel strength.

"Normal" serum concentrations are vague. In part, because there is no "optimal" serum fluoride concentration, and no "optimal" tooth fluoride concentration. Teeth with and without dental caries have the same range of fluoride concentrations. The CDC suggests, *"Normal serum fluoride levels are <20 mcg/L (**0.02 ppm**) but varies substantially. . . ."*[5] We will see below, 0.02 ppm serum fluoride is not protective. Researchers have reported various serum fluoride concentrations in studies for their "controls." It is not unusual for studies which report harm to have controls assuming "normal" with fluoride serum concentrations higher than 0.02 ppm.

Taves ('66)	normal <0.013 ppm
Sowers	controls 0.05 ppm (4th quartile)
Sandhu	controls 0.042 ppm and tumors at 0.072 ppm (Xiang 0.064 ppm)
Zang	controls 0.04 ppm and 8 IQ loss 0.08 ppm
Rathe	controls 0.025 ppm and stones at 0.12 ppm
Hossney Mother's Milk	0.000 most samples - none detected

If controls had been <0.02 ppm, greater significance might have been reported.

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[4] Nakamoto T, Rawls HR. Fluoride Exposure in Early Life as the Possible Root Cause of Disease In Later Life. J Clin Pediatr Dent. 2018;42(5):325-330. doi: 10.17796/1053-4625-42.5.1. Epub 2018 May 15. PMID: 29763350.

[5] http://www.bt.cdc.gov/agent/sulfurylfluoride/casedef.asp

Keep in mind, birth control has efficacy at parts per billion. We report fluoride here in parts per million.

II. BIAS OF FLUORIDE EFFICACY

Bias sneaks into research and evaluations of research in several forms. Our nominations for cancer and neurologic harm provided a few studies on fluoride's lack of benefit and should be reviewed. A humble attitude should be taken, remembering "our knowledge is finite, our ignorance infinite."

Ben Goldacre suggests,[6] "Medicine shouldn't be about authority, and the most important question anyone can ask on any claim is simple: 'how do you know?'" Fluoridation of public water is a web of guesses, assumptions and beliefs. Healthcare is littered with the use of treatments that are based on habit, firmly held beliefs and policy rather than evidence. Several medical treatments and research studies were started in the 40's and 50's which lacked scientific rigor evaluating risks, such as artificial fluoridation, thalidomide, and the US Public Health Service Tuskegee experiments on syphilis,[7] Vioxx, Avandia, Herceptin, diethylstilbestrol, are further recent examples.

Another bias is the "natural" ebb and flow of diseases and natural resolution of disease. Dentists seldom see dental caries resolve on their own. If we see caries, we treat. Dentists tend to approach prevention with the same arbitrary mind set. However, prevention and good health are frustratingly less in our control and arbitrary than dental treatment, and less lucrative. Comparing developed countries finds caries have been reduced the same amount regardless of fluoridation. Fair tests, prospective RCT studies of efficacy need to be done rather than assumptions. OHAT must not assume fluoride ingestion mitigates dental caries. RCT studies are possible.

> "Our many errors show that the practice of causal inference. . . remains an art. Although to assist us, we have acquired analytic techniques, statistical methods and conventions, and logical criteria, ultimately the conclusions we reach are a matter of judgement."[8]

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[6] http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0050892/pdf/TOC.pdf "Testing Treatments Better Research For Better Healthcare, 2nd Ed. Imogen Evans et al. 2011.

[7] http://www.tuskegee.edu/about_us/centers_of_excellence/bioethics_center/about_the_usphs_syphilis_study.aspx

[8] Susser M. *Causal thinking in the health sciences,* Oxford: Oxford University Press, 1983. As quoted in "Testing Treatments Better Research For Better Healthcare, 2nd Ed.

The NRC (2006) review of fluoride in water used a “weight of evidence” approach. Without any prospective RCT studies, a “weight of evidence” approach is reasonable.

Patients of healthcare should be participants rather than recipients. Doctors and public health professionals are in error when they attempt to dispense health through chemistry under police powers. Professionals are more effective for good overall health when they dispense information for collaboration in better health. “Education, not Fluoridation.”

The assumption of ingested fluoride’s efficacy has biased public health policy and scientific evaluation. We have misled ourselves and need fair tests of the evidence. Studies funded by those with vested interests are four times more likely to have a positive result. Many desire miracle cures. The marketing claim of fluoride “preventing” caries is just marketing. If ingested fluoride has any benefit, the term mitigating, rather than “preventing” would be more appropriate.

The CDC funded (Caution: vested interest and potential bias) a 2015 Cochrane study[9] on the efficacy of fluoridation. The Cochrane study includes:

> “Although these results indicate that water fluoridation is effective at reducing levels of tooth decay in children’s baby and permanent teeth, the applicability of the results to current lifestyles is unclear because the majority of the studies were conducted before fluoride toothpastes and the other preventative measures were widely used in many communities around the world.”
>
> “There was insufficient information available to find out whether the introduction of a water fluoridation programme changed existing differences in tooth decay across socioeconomic groups.”
>
> “There was insufficient information available to understand the effect of stopping water fluoridation programmes on tooth decay.”
>
> “No studies met the review’s inclusion criteria that investigated the effectiveness of water fluoridation for preventing tooth decay in adults, rather than children.”

The Cochrane report should have used only RCT studies. But since there are none, the best available where are prior to fluoride toothpaste and other preventive measures. The lack of quality studies for the only mass medication should sound the alarm. Yes, they threw bones to everyone, supporting the funders of their study, the CDC, by saying fluoridation is “effective” and yet support most developed countries which do not

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[9] Iheozor-Ejiofor Z, Worthington HV, Walsh T, O’Malley L, Clarkson JE, Macey R, Alam R, Tugwell P, Welch V, Glenny A, Water fluoridation to prevent tooth decay, Cochrane Review, June 18, 2015

fluoridate by suggesting “applicability” is unclear. . . which scientifically means what? And, Cochrane used relative percentages rather than absolute percentage. In other words, a 25% relative percentage sounds bigger than a <1% absolute percentage. A decrease from two cavities to 1.5 cavities is a relative 25% decrease. Out of 128 possible cavities, a decrease of half a cavity is less than an absolute 1% decrease.

The Cochrane (2015) study is consistent with the FDA withdrawing approval of ingested fluoride supplements in 1975, for lack of evidence of efficacy.

For decades, calls for high quality research have been made and to date not one has been published. Proponents of fluoride ingestion have claimed RCT studies are not possible, a poor excuse. Some communities such as in Alaska have water trucked to them and these could be studied. The greatest obstacle for approval of an RCT study might be acceptance by a human studies ethics review board. And if a controlled study is unethical, the same act as policy is no more ethical. *“Absence of evidence is not evidence of absence or evidence of safety.”*

CDC: “Ingestion of fluoride is not likely to reduce tooth decay.”[10]

CDC: “. . . fluoride prevents dental caries predominately after eruption of the tooth into the mouth, and its actions primarily are topical for both adults and children…”[11]

“**Systemic Fluoride has theoretical benefit while the enamel is developing, up to age 6-8.**”[12]

It makes no sense to medicate everyone with artificially fluoridated water to theoretically benefit about 10% of the population while 41% of children have dental fluorosis, a biomarker of excess fluoride exposure, for a non contagious almost never lethal disease, without patient consent.

Dental caries is not the result of inadequate fluoride ingestion and no physiologic process requires fluoride. For those wishing to ingest fluoride, other sources of fluoride ingestion (such as toothpaste) are available.

PERSPECTIVE: The EPA’s proposed RfD will increase from 0.06 mg/kg/day to **0.08** mg/kg/day. In other words, the EPA is doing the opposite of the NRC recommendation. The NRC (2006 p. 222) reported: “Impaired glucose tolerance in humans has been reported in separate studies at fluoride intakes of **0.07**-0.4 mg/kg/day, . . . The primary mechanism appears to involve inhibition of insulin production.” Mother’s milk has mean dosage of **<0.001 mg/kg/day.**

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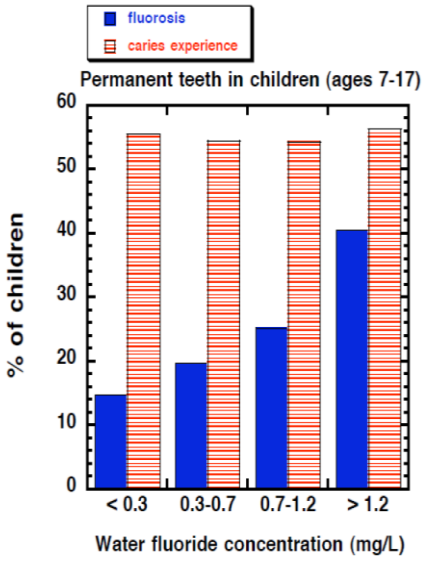
[10] (1999). Achievements in Public Health, 1900-1999: Fluoridation of Drinking Water to Prevent Dental Caries. MMWR, 48(41); 933-940, October 22

[11] IBID

[12] NRC 2006 & HHS HTSDR 2003 p 9

Vida & Kumar (2009) *"CONCLUSION: The results of this study suggest that teeth with fluorosis were more resistant to caries in U.S. schoolchildren than were teeth without fluorosis. Our results highlight the need for those considering policies regarding reduction of fluoride exposure to take into consideration the caries-preventive benefits associated with milder forms of enamel fluorosis."*

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



However, graphing lida and Kumar data demonstrates dental fluorosis does increase with more fluoride, but a discouragingly almost undetectable caries difference, well within the effects of confounding factors. Slightly more fluoride increases caries above the low fluoride. Risks increase, benefit is negligible.

Most studies evaluating the risks of fluoride are animal studies and use fluoride at higher concentrations than water fluoridation. Humans are significantly more sensitive to fluoride than rodents and an uncertainty factor of 100 is recommended. As a rough estimate, any study using 100 ppm fluoride or less on rodents raises concerns for humans (fluoridated water represents about half of human fluoride exposure).

A number of studies are published each year on fluoride's harm and studies provided here are not a definitive list. The reader should use judgment to put the weight of significance to each study and look the study up to read the full study. Abstracts often show bias.

Vandenberg et al. (2012)[13] included sodium fluoride in a list of endocrine disrupting chemicals (EDCs) with low-dose effects. They noted the EDC action of sodium fluoride as: "Inhibits insulin secretion, PTH, TH." The Vanderberg et al. paper was cited in a larger report, Science of Endocrine Disrupting Chemicals – 2012, co-published in January 2013 by the United Nations Environment Programme and the World Health Organization – see page 13

[13] Laura N. Vandenberg, Theo Colborn, Tyrone B. Hayes, Jerrold J. Heindel, David R. Jacobs, Jr., Duk-Hee Lee, Toshi Shioda, Ana M. Soto, Frederick S. vom Saal, Wade V. Welshons, R. Thomas Zoeller, and John Peterson Myers Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses. Endocrine Reviews. First published ahead of print March 14, 2012 as doi:10.1210/er.2011-1050

III. NATIONAL RESEARCH COUNCIL 2006 "FLUORIDE IN DRINKING WATER: A SCIENTIFIC REVIEW OF EPA'S STANDARDS"

NRC (2006) report, in part, is included in sections here. Their review, although historic, is still the most definitive on the relationship between fluoride and the endocrine system. This section is quoted directly from the NRC (2006) report, starting page 214.

"OTHER ENDOCRINE ORGANS

> "The effects of fluoride exposure have been examined for several other endocrine organs, including the adrenals, the pancreas, and the pituitary (for details, see Appendix E, Tables E-16 and E-17). Effects observed in animals include changes in organ weight, morphological changes in tissues, increased mitotic activity, decreased concentrations of pituitary hormones, depressed glucose utilization, elevated serum glucose, and elevated insulin-like growth factor-1 (IGF-1). Effects reported in humans include "endocrine disturbances," impaired glucose tolerance, and elevated concentrations of pituitary hormones. Studies of the effects of fluoride on glucose metabolism and in diabetic animals are discussed below; information on other effects is extremely limited.

"Animal Studies (Diabetic Animals)

> "Two studies have examined the effects of fluoride exposure in diabetic rats. In the first study, Dunipace et al. (1996) compared male Zucker fatty diabetic rats and Zucker age-matched controls given drinking water with fluoride at 5, 15, or 50 mg/L. [These fluoride intakes were considered to be equivalent to intakes by humans of 1, 3, and 10 mg/L (Dunipace et al. 1996).] For the physiological, biochemical, and genetic variables that were monitored, no "measurable adverse effects" were noted. Statistically significant differences with respect to fluoride intake (as opposed to differences between normal and diabetic animals) were observed only for diabetic rats with fluoride at 50 mg/L. No endocrinological parameters (e.g., PTH) were measured. Dunipace et al. (1996) reported that fluoride intake, excretion, and balance were generally similar in this study and in a previous study with Sprague-Dawley rats but that there were "strain-specific differences in fluoride sensitivity"; these differences were not defined or explained. The Zucker fatty diabetic rat is considered to be an animal model for human Type II (noninsulin-dependent) diabetes mellitus, although the diabetic rats in this study did not experience renal insufficiency, and the study was terminated before an age that might be more comparable to ages associated with late-onset diabetes and diabetic complications in humans. The authors concluded that the diabetic rats "were not at increased risk of fluorosis," even though femoral fluoride concentrations (2,700-9,500 µg/g in ash for diabetic rats given fluoride at 15 or 50 mg/L versus 2,500-3,600 in normal rats given fluoride at 50 mg/L) were in the range associated with fluorosis in humans and exceeded concentrations of bone fluoride associated with decreased bone strength in rabbits (6,500-8,000 ppm in ash; Turner et al. 1997); no basis for their conclusion was given.

“In the second study, Boros et al. (1998) compared the effects of fluoride at 10 mg/L in drinking water for 3 weeks on young female rats (Charles River, Wistar), either normal (nondiabetic) or with streptozotocin-induced, untreated diabetes. An additional group of normal rats was given an amount of fluoride in drinking water corresponding to the fluoride intake by the diabetic rats (up to about 3 mg/day per rat). Both feed and water consumption increased significantly in the diabetic rats (with and without fluoridated water); water consumption was significantly higher in the diabetic rats on fluoridated water than in those on nonfluoridated water. Fasting blood glucose concentrations were increased significantly in both diabetic groups, but more so in the group on fluoridated water. Fluoride treatment of nondiabetic animals did not cause any significant alteration in blood glucose concentrations. Plasma fluoride was higher, and bone fluoride was lower, in diabetic than in nondiabetic animals given the same amount of fluoride, indicating lower deposition of fluoride into bone and lower renal clearance of fluoride in the diabetic animals. The increased kidney weight found in diabetic animals on nonfluoridated water was not seen in the fluoride-treated diabetic animals. Additional biochemical and hormonal parameters were not measured.

“In contrast to the Zucker fatty diabetic rats in the study by Dunipace et al. (1996), the streptozotocin-induced diabetic rats in this study (Boros et al., 1998) provide an animal model considered representative of Type I (insulin-dependent) diabetes mellitus in humans. In these rats, the general severity of the diabetes (blood glucose concentrations, kidney function, weight loss) was worse in animals given fluoride at 10 mg/L in their drinking water. In both types of diabetic rats, fluoride intake was very high because of the several-fold increase in water consumption, and corresponding plasma, soft tissue, and bone fluoride concentrations were elevated accordingly. Thus, any health effects related to plasma or bone fluoride concentrations, for example, would be expected to occur in animals or humans with uncontrolled (or inadequately controlled) diabetes at lower fluoride concentrations in drinking water than for nondiabetics, because of the elevated water intakes. In addition, the results reported by Boros et al. (1998) suggested that, for some situations (e.g., diabetes in which kidney function is compromised), the severity of the diabetes could be increased with increasing fluoride exposure.

“Animal Studies (Normal Animals)

“Turner et al. (1997) reported a 17% increase in serum glucose in female rabbits given fluoride in drinking water at 100 mg/L for 6 months. IGF-1 was also significantly increased (40%) in these rabbits, but other regulators of serum glucose, such as insulin, were not measured. The authors suggested that IGF-1 concentrations might have changed in response to changes in serum glucose concentrations. Dunipace et al. (1995, 1998) found no significant differences with chronic fluoride treatment in mean blood glucose concentrations in rats; specific data by treatment group were not reported, and parameters such as insulin and IGF-1 were not measured.

"Suketa et al. (1985) and Grucka-Mamczar et al. (2005) have reported increases in blood glucose concentrations following intraperitoneal injections of NaF; Suketa et al. (1985) attributed these increases to fluoride stimulation of adrenal function. Rigalli et al. (1990, 1992, 1995), in experiments with rats, reported decreases in insulin, increases in plasma glucose, and disturbance of glucose tolerance associated with increased plasma fluoride concentrations. The effect of high plasma fluoride (0.1-0.3 mg/L) appeared to be transient, and the decreased response to a glucose challenge occurred only when fluoride was administered before (as opposed to together with or immediately after) the glucose administration (Rigalli et al. 1990). In chronic exposures, effects on glucose metabolism occurred when plasma fluoride concentrations exceeded 0.1 mg/L (5 µmol/L) (Rigalli et al. 1992, 1995). The in vivo effect appeared to be one of inhibition of insulin secretion rather than one of insulin-receptor interaction (Rigalli et al. 1990). Insulin secretion (both basal and glucose-stimulated) by isolated islets of Langerhans in vitro was also inhibited as a function of fluoride concentrations (Rigalli et al. 1990, 1995). Rigalli et al. (1990) pointed out that recommended plasma fluoride concentrations for treatment of osteoporosis are similar to those shown to affect insulin secretion.

"Human Studies

"Jackson et al. (1994) reported no differences in mean fasting blood glucose concentrations between osteoporosis patients treated with fluoride and untreated controls, although 3 of 25 treated individuals had values outside the normal range (versus 1 of 38 controls). No significant differences were found between groups of older adults with different fluoride concentrations in drinking water in studies in China (Li et al. 1995; subjects described as "healthy" adults) and the United States (Jackson et al. 1997), and all mean values were within normal ranges. [In the study by Jackson et al. (1997), samples were nonfasting; in the study by Li et al. (1995), it is not clear whether samples were fasting or nonfasting.] Glucose tolerance tests were not conducted in these studies.

"Trivedi et al. (1993) reported impaired glucose tolerance in 40% of young adults with endemic fluorosis, with fasting serum glucose concentrations related to serum fluoride concentrations; the impaired glucose tolerance was reversed after 6 months of drinking water with "acceptable" fluoride concentrations (<1 mg/L). It is not clear whether individuals with elevated serum fluoride and impaired glucose tolerance had the highest fluoride intakes of the group with endemic fluorosis or a greater susceptibility than the others to the effects of fluoride. For all 25 endemic fluorosis patients examined, a significant positive correlation between serum fluoride and fasting serum immunoreactive insulin (IRI) was observed, along with a significant negative correlation between serum fluoride and fasting glucose/insulin ratio (Trivedi et al. 1993).

"The finding of increased IRI contrasts with findings of decreased insulin in humans after exposure to fluoride (Rigalli et al. 1990; de la Sota et al. 1997) and inhibition of insulin secretion by rats, both in vivo and in vitro (Rigalli et al. 1990, 1995). However, the assay for IRI used by Trivedi et al. (1993) could not distinguish between insulin

and proinsulin, and the authors suggested that the observed increases in both IRI and serum glucose indicate either biologically inactive insulin—perhaps elevated proinsulin—or insulin resistance. Inhibition of one of the prohormone convertases (the enzymes that convert proinsulin to insulin) would result in both elevated proinsulin secretion and increased blood glucose concentrations and would be consistent with the decreased insulin secretion reported by Rigalli et al. (1990, 1995) and de la Sota et al. (1997). Although Turner et al. (1997) suggested fluoride inhibition of insulin-receptor activity as a mechanism for increased blood glucose concentrations, Rigalli et al. (1990) found no difference in response to exogenous insulin in fluoride-treated versus control rats, consistent with no interference of fluoride with the insulin-receptor interaction.

“Discussion (Other Endocrine Function)

“More than one mechanism for diabetes or impaired glucose tolerance exists in humans, and a variety of responses to fluoride are in keeping with variability among strains of experimental animals and among the human population. The conclusion from the available studies is that sufficient fluoride exposure appears to bring about increases in blood glucose or impaired glucose tolerance in some individuals and to increase the severity of some types of diabetes. In general, impaired glucose metabolism appears to be associated with serum or plasma fluoride concentrations of about 0.1 mg/L or greater in both animals and humans (Rigalli et al. 1990, 1995; Trivedi et al. 1993; de al Sota et al. 1997). In addition, diabetic individuals will often have higher than normal water intake, and consequently, will have higher than normal fluoride intake for a given concentration of fluoride in drinking water. An estimated 16-20 million people in the U.S. have diabetes mellitus (Brownlee et al. 2002; Buse et al. 2002; American Diabetes Association 2004; Chapter 2); therefore, any role of fluoride exposure in the development of impaired glucose metabolism or diabetes is potentially significant.

“SUMMARY

“The major endocrine effects of fluoride exposures reported in humans include elevated TSH with altered concentrations of T3 and T4, increased calcitonin activity, increased PTH activity, secondary hyperparathyroidism, impaired glucose tolerance, and possible effects on timing of sexual maturity; similar effects have been reported in experimental animals. These effects are summarized in Tables 8-1 and 8-2, together with the approximate intakes or physiological fluoride concentrations that have been typically associated with them thus far. Table 8-2 shows that several of the effects are associated with average or typical fluoride intakes of 0.05-0.1 mg/kg/day (0.03 with iodine deficiency), others with intakes of 0.15 mg/kg/day or higher. A comparison with Chapter 2 (Tables 2-13, 2-14, and 2-15) will show that the 0.03-0.1 mg/kg/day range will be reached by persons with average exposures at fluoride concentrations of 1-4 mg/L in drinking water, especially the children. The highest intakes (> 0.1 mg/kg/d) will be reached by some individuals with high water intakes at 1 mg/L and by many or most individuals with high water intakes at 4 mg/L, as well as by young children with average exposures at 2 or 4 mg/L.

“Most of the studies cited in this chapter were designed to ascertain whether certain effects occurred (or in cases of skeletal fluorosis, to see what endocrine disturbances might be associated), not to determine the lowest exposures at which they do occur or could occur. Estimates of exposure listed in these tables and in Appendix E are, in most cases, estimates of average values for groups based on assumptions about body weight and water intake. Thus, individual responses could occur at lower or higher exposures than those listed. Although the comparisons are incomplete, similar effects are seen in humans at much lower fluoride intakes (or lower water fluoride concentrations) than in rats or mice, but at similar fluoride concentrations in blood and urine. This is in keeping with the different pharmacokinetic behavior of fluoride in rodents and in man (Chapter 3) and with the variability in intake, especially for humans.”

IV. THYROID, PARATHYROID, PANCREAS, PINEAL, ADRENAL, GONADS, ENTEROENDOCRINE, PARAGANGLIA, ANTERIOR AND POSTERIOR PITUITARY, AND PLACENTA.

NRC (2006) “In summary, evidence of several types indicates that fluoride affects normal endocrine function or response; the effects of the fluoride-induced changes vary in degree and kind in different individuals. **Fluoride is therefore an endocrine disruptor** in the broad sense of altering normal endocrine function or response. The mechanisms of action remain to be worked out and appear to include both direct and indirect mechanisms, for example, direct stimulation or inhibition of hormone secretion by interference with second messenger function, indirect stimulation or inhibition of hormone secretion by effects on things such as calcium balance, and inhibition of peripheral enzymes that are necessary for activation of the normal hormone.” (page 266). (National Research Council, 2006) (Emphasis supplied)

A. THYROID GLAND:

Metabolic active cells in the body require hormones produced by the thyroid gland, triiodothyronine (T3) and thyroxine (T4). Health consequences arise when the thyroid produces too much, or too little, of these hormones.

At relatively low doses fluoride is effective at reducing thyroid function in the hyperthyroid patients. Research confirms that (1) fluoride can exacerbate the anti-thyroid effects of iodine deficiency, (2) can cause goiter in some individuals, and (3) can alter thyroid hormone levels in a manner consistent with a general thyroid suppressant. Until the 1950s, doctors in Europe and South America prescribed fluoride for hyperthyroidism. (Merck Index 1968). Fluoride therapy did reduce thyroid activity in the treated patients. (McClaren 1969; Galletti 1958; May 1937). Clinical indications suggested 2 to 5 mg of sodium fluoride per day over several months was effective, (Galletti & Joyet 1958). Note: a person drinking 3 liters of fluoridated water at 0.7 ppm with NO other fluoride source, would receive a clinical dosage to reduce thyroid activity. A comparable proposed EPA safe dosage RfD of 0.08 mg/kg/day would exceed clinically used dosages. (0.08 mg/kg X 50 kg = 4 mg. For a 100 kg person, 0.08 mg/kg X 100 kg = 8 mg fluoride). Some ADD medications still contain fluoride.

Alterations in thyroid hormones, including reduced T3 and increased TSH, in populations exposed to elevated levels of fluoride in the workplace or in the water have been reported. (NRC 2006; Susheela 2005; Mikhailets 1996; Yao 1996; Bachinskii 1985; Yu 1985).

In **clinical hypothyroidism,** the thyroid gland fails to produce sufficient quantities of the hormones triiodothyronine (T3) and thyroxine (T4). Reduced T3 and T4 can contribute to fatigue, muscle/joint pain, depression, weight gain, menstrual disturbances, impaired

fertility, impaired memory, and inability to concentrate. When T3 and T4 levels begin to fall, the pituitary gland responds by increasing production of “Thyroid Stimulating Hormone” (TSH) as a means of getting the thyroid to produce more T3 and T4.

In **subclinical hypothyroidism,** TSH levels decrease but T3 and T4 hormones are in a normal range. Subclinical hypothyroidism in pregnant women results in reduced IQ in offspring, (Klein 2001; Haddow 1999), and a recent study in the Journal of the American Medical Association found that adults with subclinical hypothyroidism had a significantly higher rate of coronary heart disease. (Rodondi 2010).

Dental fluorosis is a poor indicator of fluoride’s effect on they thyroid gland.

Thyroid Hormone Levels Based on Severity of Dental Fluorosis (Hosur 2012).

In 2006, the NRC report on fluoride for the EPA suggested studies investigating fluoride’s impact on thyroid hormone levels have produced divergent findings, but are consistent with fluoride having an anti-thyroid effect under certain circumstances. Singh (2014 see Human Thyroid below) may in part explain the “divergent findings” because dental fluorosis is a poor indication of TSH levels (see Table 3 below). **77% with dental fluorosis and 67% without dental fluorosis had derangement in thyroid hormone levels.** Both groups had abnormal serum fluoride levels and delayed eruption. **Even Group 2 drinking 0.02 ppm-0.77 ppm fluoride in water had 50% of children with abnormal serum fluoride levels.** Note: USPHS new recommendation of 0.7 ppm, represents a 14% reduction of fluoride exposure and is not enough.

Table 3

Derangement in Thyroid hormone (FT_3 , FT_4 , TSH) levels and serum fluoride levels in children of different groups

Group	No. of cases with Derangement in Thyroid hormone (FT_3, FT_4, TSH) level	No. of children with abnormal serum fluoride level	No. of children with delayed eruption
Group1A (n = 30)	23 (77%)	29 (97%)	17 (57%)
Group1B (n = 30)	20 (67%)	30 (100%)	15 (50%)
Group 2 (n = 10)	1 (10%)	5 (50%)	0 (0%)

The most common thyroid effect associated with fluoride exposure appears to be an increase in TSH levels, with or without a corresponding effect on T3 or T4. (Susheela 2005). One of the most recent studies, for example, found a trend towards higher TSH in children based on the severity of their dental fluorosis, but without a significant effect on either T3 or T4. (Hosur 2012, see figure below). These and other findings indicate that fluoride can contribute to a subclinical, if not clinical, hypothyroid condition. It remains difficult to predict the toxic dose, however, as it appears to depend, in part, on the nutritional and health status of the individual, particularly the adequacy of iodine intake. (NRC 2006).

NRC (2006) page 218. "Thyroid Function

"Fluoride exposure in humans is associated with elevated TSH concentrations, increased goiter prevalence, and altered T4 and T3 concentrations; similar effects on T4 and T3 are reported in experimental animals, but TSH has not been measured in most studies. In animals, effects on thyroid function have been reported at fluoride doses of 3-6 mg/kg/day (some effects at 0.4-0.6 mg/kg/day) when iodine intake was adequate (Table 8-1); effects on thyroid function were more severe or occurred at lower doses when iodine intake was inadequate. In humans, effects on thyroid function were associated with fluoride exposures of 0.05-0.13 mg/kg/day when iodine intake was adequate and 0.01-0.03 mg/kg/day when iodine intake was inadequate (Table 8-2).

"Several sets of results are consistent with inhibition of deiodinase activity, but other mechanisms of action are also possible, and more than one might be operative in a given situation. In many cases, mean hormone concentrations for groups are within normal limits, but individuals may have clinically important situations. In particular, the inverse correlation between asymptomatic hypothyroidism in pregnant mothers and the IQ of the offspring (Klein et al. 2001) is a cause for concern. The recent decline in iodine intake in the United States (CDC 2002d; Larsen et al. 2002) could contribute to increased toxicity of fluoride for some individuals."

NRC (2006) Tables 8-1 and 8-2 are reproduced here.

TABLE 8-1 Summary of Major Observed Endocrine Effects of Fluoride in Experimental Animals, with Typical Associated Intakes and Physiological Fluoride Concentrations

End Point	Fluoride Intake, mg/kg/day	Fluoride in Serum or Plasma, mg/L	Fluoride in Urine, mg/L	Fluoride in Bone, ppm in ash	Key References
Altered thyroid function (altered T4 and T3 concentrations)	3-6 (lower with iodine deficiency)	NA[a]	≥ 6 (possibly ≥ 2-3)	≥2,400	Stolc and Podoba 1960; Bobek et al. 1976; Hillman et al. 1979; Guan et al. 1988; Zhao et al. 1998; Cinar and Selcuk 2005
Altered calcitonin activity	2	NA	NA	3,200-3,500[b]	Rantanen et al. 1972
Altered melatonin production; altered timing of sexual maturity	3.7	NA	NA	2,800	Luke 1997
Inhibited parathyroid function	5.4	NA	NA	NA	Rosenquist et al. 1983
Increased serum glucose; increased severity of diabetes	7-10.5	0.1-0.7[c,d]	NA	>1,000	Rigalli et al. 1990, 1992, 1995; Turner et al. 1997; Boros et al. 1998
Increased parathyroid hormone concentrations, secondary hyperparathyroidism	9-10	≥ 0.2[c]	NA	2,700-3,200	Faccini and Care 1965; Chavassieux et al. 1991

[a]Not available.
[b]ppm.
[c]Serum.
[d]Plasma.

The NRC (2006) listed several limitations of the endocrine studies. More current research has included some of these limitations. One of the limitations is the interdependence of endocrine systems. The NRC (2006) p 223. "In addition, the different endocrine organs do not function entirely separately: thyroid effects (especially elevated TSH) may be associated with parathyroid effects (Stoffer et al. 1982; Paloyan Walker et al. 1997), and glucose metabolism may be affected by thyroid or parathyroid status (e.g., McCarty and Thomas 2003; Procopio and Borretta 2003; Cettour-Rose et

al. 2005). Adverse effects in individuals might occur when hormone concentrations are still in the normal ranges for a population but are low or high for that individual (Brucker-Davis et al. 2001; Belchetz and Hammond 2003). Some investigators suggest that endocrine-disrupting chemicals could be associated with nonmonotonic dose- response curves (e.g., U-shaped or inverted-U-shaped curves resulting from the superimposition of multiple dose-response curves) and that a threshold for effects cannot be assumed (Bigsby et al. 1999; Brucker-Davis et al. 2001)."

TABLE 8-2 Summary of Major Observed Endocrine Effects of Fluoride in Humans, with Typical Associated Intakes and Physiological Fluoride Concentrations

End Point	Fluoride Intake, mg/kg/day[a]	Fluoride in Serum or Plasma, mg/L	Fluoride in Urine, mg/L	Key References
Altered thyroid function (altered T4 and/or T3 concentrations)	0.05-0.1 (0.03 with iodine deficiency)	≥0.25[a]	2.4	Bachinskii et al. 1985; Lin et al. 1991; Yang et al. 1994; Michael et al. 1996; Susheela et al. 2005
Elevated TSH concentrations	0.05-0.1 (0.03 with iodine deficiency)	≥0.25[a]	≥2	Bachinskii et al. 1985; Lin et al. 1991; Yang et al. 1994; Susheela et al. 2005
Elevated calcitonin concentrations	0.06-0.87	0.11-0.26[b]	2.2-18.5 mg/day	Teotia et al. 1978
Goiter prevalence ≥ 20%	0.07-0.13 (≥ 0.01 with iodine deficiency)	NA[c]	NA	Day and Powell-Jackson 1972; Desai et al. 1993; Jooste et al. 1999
Impaired glucose tolerance in some individuals	0.07-0.4	0.08[a] 0.1-0.3[b]	2-8	Rigalli et al. 1990, 1995; Trivedi et al. 1993; de la Sota 1997
Increased parathyroid hormone concentrations, secondary hyperparathyroidism, in some individuals	0.15-0.87	0.14-0.45[b]	3-18.5 mg/day	Juncos and Donadio 1972; Teotia and Teotia 1973; Larsen et al. 1978; Teotia et al. 1978; Duursma et al. 1987; Dandona et al. 1988; Stamp et al. 1988, 1990; Pettifor et al. 1989; Srivastava et al. 1989; Dure-Smith et al. 1996; Gupta et al. 2001

[a]Serum.
[b]Plasma.
[c]Not available.

Peckham (2015) "We found that higher levels of fluoride in drinking water provide a useful contribution for predicting prevalence of hypothyroidism. We found that practices located in the West Midlands (a wholly fluoridated area) are nearly twice as likely to report high hypothyroidism prevalence in comparison to Greater Manchester (non-fluoridated area)."

Zhang (2015)[14] (Note: although this study focused on decrease in IQ with fluoride, thyroid hormone levels were also measured.) ". . . The children's IQ, fluoride contents in drinking water (W-F), serum (S-F), and urine (U-F); serum thyroid hormone levels, COMT Val158Met polymorphism, and plasma proteomic profiling were determined. . . . In conclusion, fluoride exposure was adversely associated with children's intelligence, whereas the COMT polymorphism may increase the susceptibility to the deficits in IQ due to fluoride exposure. Moreover, the proteomic analysis can provide certain basis for identifying the early biological markers of fluorosis among children."

[14] 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.

A critical study to consider is Singh (2014) which raised serious concerns that **dental fluorosis is a poor indication of excess total fluoride exposure.** Both those with and without dental fluorosis had thyroid derangement and high serum fluoride concentrations.

Singh (2014)[15] "The study was undertaken to determine serum/urinary fluoride status and comparison of free T4, free T3 and thyroid stimulating hormone levels of 8 to 15 years old children with and without dental fluorosis living in an endemic and non-endemic fluorosis area. . . A significant relationship of water fluoride to urine and serum fluoride concentration was seen. The serum fluoride concentration also had significant relationship with thyroid hormone (FT3/FT4) and TSH concentrations. The testing of drinking water and body fluids for fluoride content, along with FT3, FT4, and TSH in children with dental fluorosis is desirable for recognizing underlying thyroid derangements and its impact on fluorosis. . . . Conclusion: The results of this study question the validity of the fluoridation of drinking water, milk, fruit juices, and salt by public health authorities and also the step taken to prevent ill effects of excess fluorine and iodine deficiencies in endemic fluorosis areas. The children with dental fluorosis living in endemic fluorosis areas may not have a frank thyroid disease due to excessive fluorine consumption but they do show thyroid disease leading to many health effect hence they require special care and attention."

And further, Singh (2014), "Group 1 included 60 male and female school children, which were equally divided into two subgroups: Group 1A (children with dental fluorosis) and Group 1B (children without dental fluorosis). Group 2 included 10 children from Sardarpura colony of Udaipur city, a non endemic area, which was taken as a control for the study samples."

Tables 1, 2, 3, and 6 of Singh (2014) are reproduced here.

Table 1: Comparing fluoride Group 1 A (dental fluorosis) and 1B (no fluorosis), with control Group 2 is consistent with other studies when urine and serum fluoride concentrations are compared with water fluoride concentrations, provided significant other sources such as fluoridated toothpastes are not in use.

[15] Singh N[1], Verma KG[2], Verma P[3], Sidhu GK[4], Sachdeva S[3]. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. Springerplus. 2014 Jan 3;3:7. doi: 10.1186/2193-1801-3-7. eCollection 2014.

Table 1

Levels of fluoride naturally ingested from drinking water and body fluids in different sample groups

Parameters	Group 1A	Group 1B	Group 2	Total
Water fluoride (WF)	1.6–5.1 ppm	1.6–5.5 ppm	0.98–1 ppm	0.98–5.5 ppm
Urine fluoride (UF)	0.24–8.9 ppm	0.4–7.79 ppm	0.19–1.01 ppm	0.24–8.9 ppm
Serum fluoride (SF)	0.02–0. 77 ppm	0.03–0.75 ppm	0.02–0.09 ppm	0.02–0.77 ppm

NOTE: The absence of dental fluorosis does not indicate lower or safe fluoride urine or serum concentrations.
NOTE: All three groups had some individuals with low serum and urine fluoride concentrations. The significant difference is those with high serum and urine fluoride concentrations.
And remember, endemic fluoride is usually CaF which is estimated at 800 times less toxic than NaF or HSF used for artificial fluoridation.

Table 2

Levels of thyroid hormones in all sample groups

Parameters	Group 1A	Group 1B	Group 2	Total
Free T_3 (FT_3)	1.1–4.39 pg/ml	1.2–4.57 pg/ml	1.90–4.13 pg/ml	1.1–4.57 pg/ml
Free T_4 (FT_4)	0.94–1.98 ng/dL	0.8–1.7 ng/dL	0.87–1.67 ng/dL	0.8–1.98 ng/dL
TSH	1.41–8.46 µIU/m	1.92–10.99 µIU/m	0.96–3.54 µIU/m	0.96–10.99 µIU/m

Table 6

Correlation analysis between fluoride content in body fluids and their effect FT3, FT4, TSH within Group 1

Parameters	Spearman rho analysis	FT_3	FT_4	TSH	WF	UF	SF
FT_3	'r'	1	-0.169	-0.252	-0.711	-0.388	-0.400
	p-value	-	0.196	0.052*	0.000**	0.002**	0.002**
FT_4	'r'	-0.169	1	-0.079	0.196	0.119	0.119
	p-value	0.196	-	0.547	0.134	0.365	0.366
TSH	'r'	-0.252	-0.079	1	0.151	0.079	0.552
	p-value	0.052*	0.547	-	0.250	0.550	0.000**
WF	'r'	-0.711	0.196	0.151	1	0.690	0.529
	p-value	0.000**	0.134	0.250	-	0.000**	0.000**
UF	'r'	-0.388	0.119	0.079	0.690	1	0.525
	p-value	0.002**	0.365	0.550	0.000**	-	0.000**
SF	'r'	-0.400	0.119	0.552	0.529	0.525	1
	p-value	0.002**	0.366	0.000**	0.000**	0.000**	-

*Correlation is significant at 0.05 levels (2 tailed), **Correlation is highly significant below 0.01 levels correlation coefficient(r).

Table 3 should be carefully considered and we graphed their Table 3 below. **Even with fluoride serum levels between 0.02 ppm and 0.09 ppm (1 ppm fluoride in water), 10% had derangement of the thyroid.** Remember, endemic fluoride is not as toxic as sodium fluoride or HFS, and second, rural villagers often use less fluoride toothpaste, dental and medical products or fluoride pesticides.

The CDC's recommendation of normal fluoride serum concentrations <0.02 ppm may not be protective and provides no margin of safety. A 0.7 ppm artificial fluoridation will not reduce serum fluoride concentrations to within CDC recommendations.

Table 3

Derangement in Thyroid hormone (FT_3, FT_4, TSH) levels and serum fluoride levels in children of different groups

Group	No. of cases with Derangement in Thyroid hormone (FT_3, FT_4, TSH) level	No. of children with abnormal serum fluoride level	No. of children with delayed eruption
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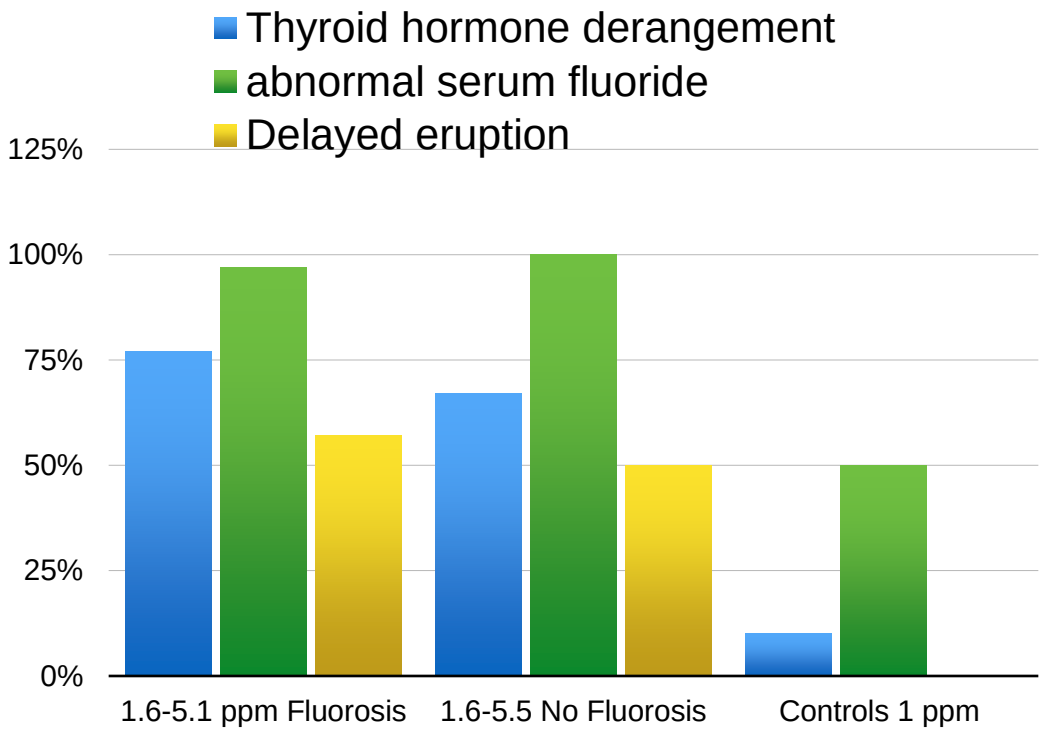

Liu (2014)[16] "In many regions, excessive fluoride and excessive iodide coexist in groundwater, which may lead to biphasic hazards to human thyroid. To explore fluoride-induced thyroid cytotoxicity and the mechanism underlying the effects of excessive iodide on fluoride-induced cytotoxicity, a thyroid cell line (Nthy-ori 3-1) was exposed to excessive fluoride and/or excessive iodide. Cell viability, lactate dehydrogenase (LDH) leakage, reactive oxygen species (ROS) formation, apoptosis, and the expression levels of inositol-requiring enzyme 1 (IRE1) pathway-related molecules were detected. Fluoride and/or iodide decreased cell viability and increased LDH leakage and apoptosis. ROS, the expression levels of glucose-regulated protein 78 (GRP78), IRE1, C/EBP homologous protein (CHOP), and spliced X-box-binding protein-1 (sXBP-1) were enhanced by fluoride or the combination of the two elements. Collectively, excessive fluoride and excessive iodide have detrimental influences on human thyroid cells. Furthermore, an antagonistic interaction between fluoride and excessive iodide exists, and cytotoxicity may be related to IRE1 pathway-induced apoptosis."

Kutlucan (2013)[17] "AIM: To compare the urine iodine, fluoride, and to measure thyroid volumes in 10-15-year-old children using ultrasonography, a gold standard in evaluating thyroid volume. . . . After puberty, echobody index in subjects with fluorosis was markedly high. Based on our results, we thought that fluorosis increases thyroid volume in children with fluorosis after puberty."

TSH is considered a "'precise and specific barometer' of thyroid status in most situations" (NRC 2006) The relationship between fluoride and elevated TSH has been found even where T3 and T4 levels remain normal, suggesting that fluoride could contribute to subclinical hypothyroidism, which is a condition of "mild thyroid failure" marked by increased TSH and normal T3/T4.

Subclinical hypothyroidism is now considered a "clinically important disorder that has adverse clinical consequences." (Gencer 2012). Several studies have found that subclinical hypothyroidism in pregnant woman was a risk factor for reduced IQ in the offspring. (Klein 2001; Haddow 1999). Although most of the more than 40 human studies evaluating fluoride and IQ did not measure TSH, those that did so reported that children with high fluoride exposures had elevated TSH levels. (Wang 2001; Yao 1996; Lin 1991). Lin reported that elevated TSH correlated with reduced IQ. TSH levels could be one of the contributing factors towards the reduced IQ reported in the studies to date.

In 2010, a study in the Journal of the American Medical Association found that adults with subclinical hypothyroidism had a significantly higher incidence of, and mortality from, coronary heart disease. (Rodondi 2010). Whether this could help explain the relationship between elevated fluoride and cardiovascular disease remains to be

[16] Liu H, Zeng Q, Cui Y, Yu L, Zhao L, Hou C, Zhang S, Zhang L, Fu G, Liu Y, Jiang C, Chen X, Wang A. The effects and underlying mechanism of excessive iodide on excessive fluoride-induced thyroid cytotoxicity. Environ Toxicol Pharmacol. 2014 Jul;38(1):332-40. doi: 10.1016/j.etap.2014.06.008. Epub 2014 Jun 27.

[17] Kutlucan A[1], Kale Koroglu B, Numan Tamer M, Aydin Y, Baltaci D, Akdogan M, Ozturk M, Vural H, Ermis F. The investigation of effects of fluorosis on thyroid volume in school-age children. Med Glas (Zenica). 2013 Feb;10(1):93-8.

determined. As reported below, one recent study (Karademir 2011) did find a relationship between fluoride exposure, thyroid levels, and cardiovascular indices, although TSH levels were not found to be elevated.

Banjo (2013) "The study investigated the role of Spirulina platensis in reversing sodium fluoride-induced thyroid, neurodevelopment and oxidative alterations in offspring of pregnant rats. . . . Fluoride-induced alterations in thyroid hormones, behaviour and increased oxidative stress. Spirulina augmented the displacement of fluoride, facilitated antioxidant formation, improved behaviour and protected Purkinje cells. Supplementing Spirulina during pregnancy could reduce the risk of fluoride toxicity in offspring."[18]

Karademir (2011)[19] "In this study we examined the deleterious effect of fluorosis on cardiovascular system including detailed ECG with dispersion analysis, echocardiography, and HRV with Holter analysis in children. We found statistically significant low T4 levels, hypocalcemia and hyponatremia, increased QT and QTc interval in children with dental fluorosis. Our results show that fluorosis might increase risk of arrhythmia indirectly, due to its hypocalcemic, hypernatremic, and hypothyroidism effects."

Ba (2009)[20] "The concentration of serum TSH of children from high fluoride and iodine area and high iodine area was higher than that of children from high fluoride area and control area. Conclusion: High fluoride and iodine increase the prevalence of goiter. High iodine increases the concentration of FT4. Fluoride can increase the concentration of FT4 under high iodine condition."

Ruiz-Pagan (2006)[21] "This study was designed to evaluate adverse health effects in adolescents from chronic exposure to various water fluoride concentrations in three communities located in Northern Mexico: Ciudad Juarez, Samalayuca, and Villa Ahumada. In these communities the fluoride concentration in water averages 0.3, 1.0, and 5.3 mg/L, respectively. The residents of Villa Ahumada have been exposed to excessive levels of fluoride in drinking water since their birth. . . . In Villa Ahumada, a significant inverse relationship was found between urine fluoride levels and stature; this association suggests that fluoride exposure may affect the teeth but also the growth of adolescents. Serum samples of these individuals showed

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[18] Banji D et al (2013) Investigation on the role of Spirulina platensis in ameliorating behavioural changes, thyroid dysfunction and oxidative stress in offspring of pregnant rats exposed to fluoride. 2013 Sep 1;140(1-2):321-31. doi: 10.1016/j.foodchem.2013.02.076. Epub 2013 Feb 28.

[19] Karademir S, et al. (2011). Effects of fluorosis on QT dispersion, heart rate variability and echocardiographic parameters in children. Anadolu Kardiyol Derg 11(2):150-55.

[20] Ba Y, et al. (2009). Effect of different fluoride and iodine concentration in drinking water on children's dental fluorosis and thyroid function. Chinese Journal of Public Health 25(8):942-43.

[21] Ruiz-Payan A. (2006). Chronic effects of fluoride on growth, blood chemistry and thyroid hormones in adolescents residing in three communities in Northern Mexico. *ETD Collection for University of Texas, El Paso.* Paper AAI3214004.
http://digitalcommons.utep.edu/dissertations/AAI3214004

elevated levels of alkaline phosphatase (ALP), potassium, magnesium, calcium, and phosphate, and decreased levels of thyroid hormone T3 and uric acid. These findings show that chronic exposure to high levels of fluoride have a definitive impact on the prevalence and severity of dental fluorosis, decreased stature, and decreased [] thyroid hormone secretion."

Susheela (2005)[22] "Although it has long been suggested that dental fluorosis is associated with IDD and thyroid dysfunction,7-9,14 this study, to our knowledge, is the first to investigate dental fluorosis in relation to TSH and the thyroid hormones FT4 and FT3, the latter now confirmed to be the biologically active thyroid hormone. As evident from the data in Table 5, deviations in thyroid hormone levels in the 49 affected children of the sample group fall into five distinct categories, which are discussed below. It is also evident that even in some of the children in the two control groups consuming "safe" water (<1.0 ppm F–), fluoride levels in their blood and urine are above current upper limits, indicating other sources of fluoride ingestion, such as from foods and beverages, dental products, drugs, air, or salt. In those children disturbances in thyroid hormone ratios are observed as well. . . . Some of the conclusions and recommendations we draw from this study are:
• Children with dental fluorosis living in endemic fluorosis areas and IDD (iodine deficiency disorder) may have thyroid derangements that require special care and attention.
• The primary cause of IDD may not always be iodine deficiency, but it might be induced by fluoride poisoning.
• Testing of drinking water and body fluids for fluoride content, along with FT3, FT4, and TSH—even in children without dental fluorosis—is desirable for recognizing thyroid derangements.
• Prevention and control of fluorosis and IDD require an integrated approach for diagnosis and patient management, contrary to prevailing practices.
• The results of this study question the validity of the fluoridation of drinking water, milk, fruit juices, and salt by public authorities."

Social (2005)[23] "In the current investigation 46.9% of the children in the [high fluoride] group have elevated TSH and normal FT4 and FT3 levels, while a similar derangement is also observed in 18.2% of the children in [the lower fluoride group]. This is our first category and is usually the first indication of thyroid dysfunction, termed sub-clinical hypothyroidism."

Cigar (2005)[24] "In this study, the serum levels of thyroxine (T4), triiodothyronine (T3), and protein-bound iodine (PBI) in the control cows were in the normal range of

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[22] AK Susheela, M Bhatnagar, K.Vig, NK Mondald, EXCESS FLUORIDE INGESTION AND THYROID HORMONE DERANGEMENTS IN CHILDREN LIVING IN DELHI, INDIA. Fluoride 2005;38(2):151–161 Research report 151

[23] Susheela AK, et al. (2005). Excess fluoride ingestion and thyroid hormone derangements in children living in New Delhi, India. Fluoride 38(2):98-108.

[24] Cinar A, Selcuk M. (2005). Effects of chronic fluorosis on thyroxine, triiodothyronine, and protein-bound iodine in cows. Fluoride 38(1):65-68.

healthy cows, but they were significantly lower ($p<0.05$) in the fluorotic cows. These findings are consistent with the results of research with sheep, calves, cattle, and rats. . . . On the other hand, Choubisa reported that none of a group of fluorotic domestic animals exhibited any apparent evidence of hypothyroidism, stunted growth, [or] low milk production . . . . In our view, the reason for decreased levels of T4, T3, and PBI in our cows with chronic fluorosis might be due to: 1) inhibition of the absorption of the iodine and some amino acids (e.g., tyrosine) in the gastrointestinal tract, 2) insufficient synthesis and secretion of thyroglobulin and oxidized iodides from the thyroid glands, 3) low levels of bioavailable iodine in the Tendurek Mountain region."

"Wang (2001)[25] In conclusion, high iodine and high fluorine in the drinking water have, to some extent, effects on children's intelligence and thyroid function."[26]Wang (2001) "TSH value was obviously higher than the control point, indicating that, under high iodine and high fluorine condition, T3 and T4 secreted by the thyroid are in the normal range, while TSH value secreted by the pituitary clearly increased. This is probably because high iodine and high fluorine suppress the synthesis and secretion of the thyroid peroxidase and thyroid hormones . . . . The body accelerates the Hypothalamic TSH secretion by negative feedback regulation, thus increasing the secretion of TSH, stimulating the composition of T3 and T4 of the thyroid. As a result, the TSH in the peripheral blood circulation is high while T3 and T4 are not clearly reduced."

Liu (2001) "Objective: To investigate the effects of fluoride on thyroid structure in chicks.. . . Conclusions Fluoride can seriously damage thyroid structure . During the earlier stage, fluoride can induce thyroid atrophia, however, during the later stage, it can induce thyroid enlargement which is nodular and colloid goiter."[27]

Wan (1999)[28] [Objective: To study the significant test of diagnosing endemic fluorosis. Methods Twenty one routine and biochemical marks of blood and urine from 600 cases of the patients with different degree endemic fluorosis were determined and analysed. Results . . . The average of T3 and T4 were lower than the reference value, particularly in those with moderate and severe stages of the disease. Conclusions The RBC, Hb, serum calcium,phosphorus, AKP, urinary calcium, globulin, T3 and T4 were signifiant diagnostic indicators of endemic fluorosis.]

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[25] Wang X, et al. (2001). Effects of high iodine and high fluorine on children's intelligence and thyroid function. *Chinese Journal of Endemiology* 20(4):288-90.

[26] Wang X, et al. (2001). Effects of high iodine and high fluorine on children's intelligence and thyroid function. Chinese Journal of Endemiology 20(4):288-90.

[27] Liu GY, et al. (2001). Effects of fluoride on thyroid structure in chicks. Chinese Journal of Endemiology.

[28] Wan G, et al. (2001). Determination and analysis on multimark of test of patients with endemic fluorosis. Chinese Journal of Endemiology 20(2):137-39.

Xiaoli (1999)[29] [In a group of 8-12 year old children living in an endemic fluorosis area in China, TSH levels were significantly elevated, while T4 levels were significantly decreased and T3 levels significantly increased.]

Yao (1996)[30] "The TSH level is a sensitive index which both reflects the state of the body's thyroid function, and screens the level of iodine (lack thereof) in a population. TSH is also a sensitive indicator in terms of making timely discoveries of people suffering from poor thyroid function or below-average intelligence. The results from this test show that TSH values of children with dental fluorosis from the two endemic areas is at a remarkably higher level than those from the non-endemic area. Children from the endemic areas were also found to have a lower level of intelligence than the non-endemic group. The heavier the level/concentration of fluoride found in the region, the more significant the difference in the results."

Mikhail's (1996)[31] "Conclusions: 1. Abnormalities in the thyroid function characterized by a decreased iodine absorption function of the thyroid, a low level T3 syndrome, and a slight increase of the TSH level are observed in cases of chronic fluorine intoxication in the industrial workers. 2. The observed changes progressed with the increase of the time of exposure to fluorides and a more advanced disease stage. 3. The highest frequency of occurrence of the low level T3 syndrome was observed in workers with chronic fluoride intoxication including TPP (toxic liver damage). 4. The lowered iodine absorption function of the thyroid and/or the low level T3 syndrome can serve as diagnostic signs of chronic fluorine intoxication. 5. The decrease in the T3 level most probably occurs due to the disrupted conversion of T4 to T3 at the cell- target level. The disruption of conversion may be caused by fluorine affecting the enzyme system of deiodination as well as the toxic liver damage it causes."

Shufen (1996)[32] "The levels of serum T3, T4 and TSH were analyzed in children with fluoride-aluminum combined toxicosis in the Shuicheng area of Guizhou as compared with the children without fluoride-aluminum combined toxicosis. The results showed that serum T4 content decreased in the children with fluoride aluminum combined toxicosis (103.9±15.9 nmol/L vs 150.67±16.5 nmol/L, p 0 01), but no obvious differences of serum T3 and TSH were found among total three groups. It suggests that the disorder of the thyroid function should be considered when treating the children with fluoride aluminum combined toxicosis."

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[29] Xiaoli L, et al. (1999). The detection of children's T3, T4 and TSH contents in endemic fluorosis areas. Endemic Disease Bulletin 14(1):16-17.

[30] Yao Y, et. al. (1996). Analysis on TSH and intelligence level of children with dental Fluorosis in a high fluoride area. Literature and Information on Preventive Medicine 2(1):26-27

[31] Mikhailets ND, et al. (1996). Functional state of thyroid under extended exposure to fluorides. Probl Endokrinol 42:6-9.

[32] Shufen J, et al. (1996). The change of thyroid functlon from children with fluoride aluminum combined toxicosis in Shuicheng area of Guizhou. Journal of Guiyang Medical College.

Michael (1996)[33] "While levels of thyroid stimulating hormone (TSH) and triiodothyronine (T3) did not vary, a significant increase in the thyroxine (T4) levels suggested alteration in thyroid function."

Yang (1994)[34] "An excess of fluoride and a lack of iodine in the same environment has been shown to have a marked effect on child intellectual development, causing a more significant intellectual deficit than lack of iodine alone. In our study the study group of children from the high fluoride-high iodine village area had an average IQ of 76.67±7.75, which was somewhat lower than the control (IQ 81.67 ±11.9), although the difference is not statistically significant (P > 0.05). However, as seen in Table 2, the percentage of children in the low range (16.67%) is higher in the endemic group than in the control group (10.0%), suggesting that a high iodine-high fluoride environment also has a definite negative influence on child intellectual ability."

Xu (1994)[35] "The number of children whose level of intelligence is lower is significantly increased in regions of high fluoride/iodine, regions of high fluoride only, regions of high fluoride/low iodine, against their respective comparative groups."

Lin (1991)[36] "Area A (high fluoride, low iodine) differed from area B (normal fluoride, low iodine) by having lower mean IQ, higher TSH, slightly higher 1311 uptake, and higher urinary iodine. . . . The significant ditferences in IQ among these regions suggests that fluoride can exacerbate central nervous lesions and somatic developmental disturbance caused by iodine deficiency. . . . [W]e found that 69% of the children with mental retardation had elevated TSH levels. IQ and TSH were negatively correlated. Many investigators regard an elevated TSH in the presence of normal T4 and T3 levels as evidence for hypothyroidism that is subclinical but that can still affect the development of brain and cerebral function to some degree."

Liu (1988)[37] "Endemic fluorosis is a systemic disease. We investigated the serum free fluoride, thyroid hormones and TSH concentrations in 37 cases. Significantly lowered serum T4 . . . and increased TSH were found in patients. Patients'serum T3 concentrations were not significantly different from the controls. Significant negative correlations were found between serum free fluoride concentrations and T3 concentrations or T3/T4 ratios. We propose that fluoride intoxication might decrease thyroid function and suggest the method to prevent and treat this condition."

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[33] Michael M, et al. (1996). Investigations of soft tissue function in fluorotic individuals of North Gujurat. Fluoride 29(2):63-71.

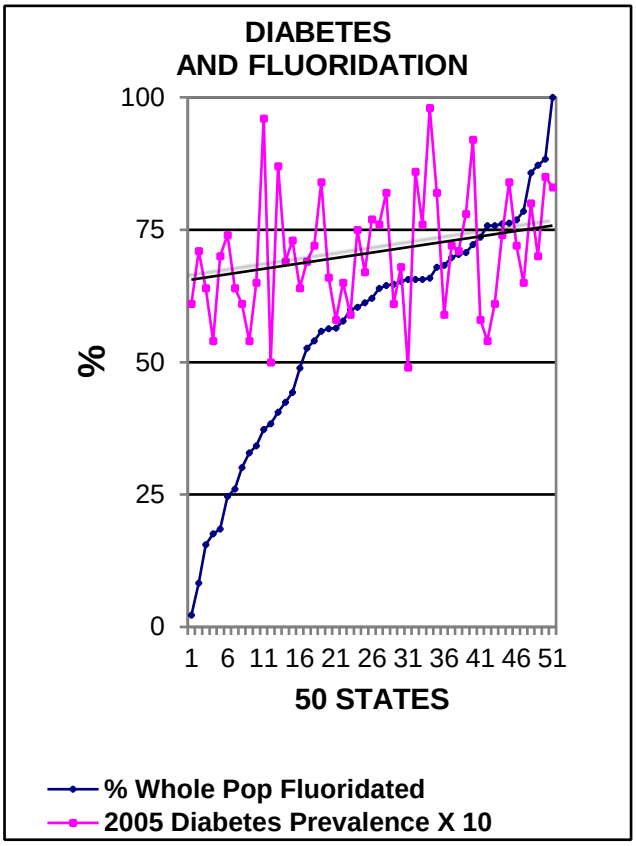
[34] Yang Y, et al. (1994). The effects of high levels of fluoride and iodine on intellectual ability and the metabolism of fluoride and iodine. Chinese Journal of Epidemiology 15(4):296-98 (republished in Fluoride 2008; 41:336-339).

[35] Xu Y, et al. (1994). The effect of fluorine on the level of intelligence in children. *Endemic Disease Bulletin* 9(2):83-84.

[36] Lin F; et al (1991). The relationship of a low-iodine and high-fluoride environment to subclinical cretinism in Xinjiang. *Endemic Disease Bulletin* 6(2):62-67 (republished in *Iodine Deficiency Disorder Newsletter* Vol. 7(3):24-25).

[37] Liu Z, et al. (1988). An investigation on the serum thyroid hormones and fluoride concentrations in patients with endemic fluorosis. Chinese Journal of Endemiology 7(4):216-18. [Article in Chinese with English summary]

Bachinskii (1985)[38] "The ingestion of drinking water with high concentrations of fluoride (122 +/- 5 micromoles per liter) leads, in healthy people, to stress of the functional status of the pituitary-thyroid system, as evidenced by a reduction in the concentration of T3, an increase in the production (by the hypothalamus) of TSH in the serum, and a more avid uptake of I131 by the thyroid tissue. This permits us to classify the excessive accumulation of fluorine in the body as a risk factor providing a basis for the development of thyroid dysfunction."



Yu (1985)[39] "A study on the serum T4, T3 and TSH levels was performed in 27 patients with chronic skeletal fluorosis and the data obtained were compared with those of 20 health persons. The results showed that serum T4 in the patients was lower than in the controls and TSH was higher, while serum T3 showed no significant difference. There was no goiter found in the patients. These data indicate that fluorine may reduce serum T4 by interfering [with] thyroid function.The increase of TSH secretion is the consequence stimulated by a feedback mechanism but no proliferation and enlargement of the thyroid gland resulted . . . ."

Graphing the 50 US states ranked on the percentage of the whole population fluoridated and plotting their respective rate of diabetes (X10)[40] provides this graph, perhaps a 10% increase in diabetes. Remember, fluoridated water represents only about half of fluoride exposure.

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[38] Bachinskii PP et al. 1985. Action of the body fluorine of healthy persons and thyroidopathy patients on the function of hypophyseal-thyroid the system. Probl Endokrinol (Mosk) 31(6):25-9. [Article in Russian, translated into English]

[39] Yu Y. (1985). Study on serum T4, T3, and TSH levels in patients with chronic skeletal fluorosis. Chinese Journal of Endemiology 4(3):242-43.

[40] Note: In order to view the data on one graph, the percentage of fluoridated in each state is correct but the percentage of diabetes is increased by 10 fold. In other words, 75 is actually 7.5% for diabetes and 75% for fluoridation. Source of data: http://apps.nccd.cdc.gov/nohss/FluoridationV.asp
http://www.unitedhealthfoundation.com/shr2005/components/obesity.html
http://pubs.usgs.gov/circ/2004/circ1268/htdocs/table05.html

Treatment

ANIMAL TREATMENT Sarkar (2014) Resveratrol (3,4,5-trihydroxystilbene), a polyphenol and well-known natural antioxidant has been evaluated for its protective effect against fluoride-induced metabolic dysfunctions in rat thyroid gland. . .Resveratrol supplementation in fluoride-exposed animals appreciably prevented metabolic toxicity caused by fluoride and restored both functional status and ultra-structural organization of the thyroid gland towards normalcy. This study first establishes the therapeutic efficacy of resveratrol as a natural antioxidant in thyroprotection against toxic insult caused by fluoride."[41]

B. PARATHYROID GLAND

Wang (2015)[42] "Parathyroid hormone (PTH), PTH-related peptide (PTHrP), and calcium-sensing receptor (CaSR) play important roles in maintaining calcium homeostasis. Here, we study the effect of fluoride on expression of PTH, PTHrP, and CaSR both in vitro and in vivo. MC3T3-E1 cells and Sprague-Dawley rats were treated with different concentrations of fluoride. Then, the free calcium ion concentration in cell culture supernatant and serum were measured by biochemical analyzer. The expression of PTH, PTHrP, and CaSR was analyzed by qRT-PCR and Western blot. We found that the low dose of fluoride increased ionized calcium (i[Ca(2+)]) and the high dose of fluoride decreased i[Ca(2+)] in cell culture supernatant. The low dose of fluoride inhibited the PTH and PTHrP expression in MC3T3-E1 cells. The high dose of fluoride improved the PTHrP expression in MC3T3-E1 cells. Interestingly, we found that NaF decreased serum i[Ca(2+)] in rats.

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[41] Sarkar C[1], Pal S. Ameliorative effect of resveratrol against fluoride-induced alteration of thyroid function in male wistar rats. Biol Trace Elem Res. 2014 Dec;162(1-3):278-87. doi: 10.1007/s12011-014-0108-3. Epub 2014 Aug 28.

[42]Wang Y1, Duan XQ, Zhao ZT, Zhang XY, Wang H, Liu DW, Li GS, Jing L., Fluoride Affects Calcium Homeostasis by Regulating Parathyroid Hormone, PTH-Related Peptide, and Calcium-Sensing Receptor Expression. Biol Trace Elem Res. 2015 Jun;165(2):159-66. doi: 10.1007/s12011-015-0245-3. Epub 2015 Feb 3.

Fluoride increased CaSR expression at both messenger RNA (mRNA) and protein levels in MC3T3-E1 cells and rats. The expression of PTHrP protein was inhibited by fluoride in rats fed regular diet and was increased by fluoride in rats fed low-calcium diet. Fluoride also increased the expression of PTH, NF-kappaB ligand (RANKL), and osteoprotegerin (OPG) in rats. The ratio of RANKL/OPG in rats fed low-calcium food in presence or absence of fluoride was significantly increased. These results indicated that fluoride might be able to affect calcium homeostasis by regulating PTH, PTHrP, and CaSR."

Shashi (2013)[43] **Abstract:** The present study assessed the effect of fluoride on parathyroid function in 860 patients (mean age 32.50±10.50) affected with skeletal fluorosis, selected randomly from endemic fluorotic areas of district Bathinda, Punjab, India. The fluoride content in water sources was found to vary from 0.68-15.78 mg/L in study areas. Hence, the study areas were categorized as five different groups Control (0.68- 1.00 mg/L), A-I (1.01-4.00 mg/L), A-II (4.01-8.00 mg/L), A-III (8.01-12.00 mg/L) and A-IV (12.01-16.00 mg/L). An age and sex matched group of 140 control subjects without skeletal fluorosis were also included. The functional activity of the parathyroid was measured by radio immuno assay of parathyroid hormone (PTH). The biochemical estimations were made for serum and urinary fluoride, serum calcium, phosphorus, calcitonin and alkaline phosphatase (ALKP). The results revealed that level of serum and urinary fluoride was significantly ($p<0.001$) higher in fluorotic patients in comparison to control. The serum PTH, calcitonin and activity of ALKP was significantly ($P<0.001$) elevated in fluorotic patients. Significant ($P<0.05$) hypocalcaemia was observed in study group A-I and A-II and elevation in group A-IV. However, the alterations in calcium level in group A-III was statistically non significant. Hyperphosphatemia ($P<0.001$) was also observed in patients of fluorosis. Pearson's bivariate correlation showed positive correlation between water F vs serum F ($r= 0.98$, $P<0.001$), serum F vs PTH ($r= 0.97$, $P<0.007$), serum F vs calcitonin ($r=0.80$, $P<0.01$) and serum F vs ALKP ($r=0.93$, $P<0.02$). Negative correlation was noted between serum and urinary concentration of fluoride. When the serum fluoride concentration was increased the corresponding urinary fluoride excretion declined along with the advancing age. It may be concluded that high fluoride ingestion has a definite relation with increased calcitonin concentration, which may be the major cause of hypocalcemia in fluorotic patients, which may further leads to the increased parathyroid function i.e raised PTH levels in the serum to maintain serum calcium levels and may have a role in toxic manifestations of clinical and skeletal fluorosis."

Puranik (2013)[44] "Objective: This study investigated fluoride's effects on iPTH secretion

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[43] *A Shashi and Swati Singla.* **Parathyroid Function in Osteofluorosis,** World Journal of Medical Sciences 8 (1): 67-73, 2013 ISSN 1817-3055 © IDOSI Publications, 2013**,** DOI: 10.5829/idosi.wjms.2013.8.1.72168 http://www.idosi.org/wjms/8(1)13/11.pdf

[44] *Puranik, Chaitanya Prakash,* Ph.D., **Effect of Fluoride on Parathyroid Hormone Secretion,** Dissertation. **THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL**, 2013, 129 pages; 3606754

and its underlying mechanism. . . . Conclusion: Fluoride modulates iPTH secretion in vitro and in vivo. However, Fluoride's action on the parathyroid gland is not mediated through CASR. While fluoride's effects, in vitro, were equivalent between the two mouse strains, early strain-dependent effect on iPTH secretion was observed in vivo. Difference in fluoride-mediated gene expression in C3H and B6 suggests an underlying difference in physiologic handling of fluoride by the two strains."

Peng (2013)[45] "Chronic exposure to combined fluoride and arsenic continues to be a major public health problem worldwide, affecting thousands of people. In recent years, more and more researchers began to focus on the interaction between the fluorine and the arsenic. In this study, the selected investigation site was located in China. The study group was selected from people living in fluoride-arsenic polluted areas due to burning coal. The total number of participants was 196; including the fluoride-arsenic anomaly group (130) and the fluoride-arsenic normal group (63). By observing the changes in gene and protein expression of PTH/PKA/AP1 signaling pathway, the results show that fluoride can increase the expression levels of PTH, PKA, and AP1, but arsenic can only affect the expression of AP1; fluoride and arsenic have an interaction on the expression of AP1. Further study found that fluoride and arsenic can affect the mRNA expression level of c-fos gene (AP1 family members), and have an interaction on the expression of c-fos, but not c-jun. The results indicate that PTH/PKA/AP1 signaling pathway may play an important role in bone toxicity of fluoride. Arsenic can affect the expression of c-fos, thereby affecting the expression of transcription factor AP1, indirectly involved in fluoride-induced bone toxicity."

Gutowska (2013)[46] "Chronic long-term exposure to high levels of fluoride leads to fluorosis, manifested by skeletal fluorosis and damage to internal organs, including kidneys, liver, parathyroid glands, and brain. Excess fluoride can also cause DNA damage, trigger apoptosis, and change cell cycle. The effect of fluoride may be exacerbated by lead (Pb), a potent inhibitor of many enzymes and a factor causing apoptosis, still present in the environment in excessive amounts. Therefore, in this study, we investigated the effects of sodium fluoride (NaF) and/or lead acetate (PbAc) on development of apoptosis, cell vitality, and proliferation in the liver cell line HepG2. We examined hepatocytes from the liver cell line HepG2, incubated for 48 h with NaF, PbAc, and their mixture (NaF + PbAc), and used for measuring apoptosis, index of proliferation, and vitality of cells. Incubation of the hepatocytes with NaF or PbAc increased apoptosis, more when fluoride and Pb were used simultaneously. Vitality of the cells depended on the compound used and its concentration. Proliferation slightly increased and then decreased in a high fluoride environment; it

[45]Zeng QB1, Xu YY1, Yu X2, Yang J2, Hong F3, Zhang AH1. Arsenic may be involved in fluoride-induced bone toxicity through PTH/PKA/AP1 signaling pathway. Environ Toxicol Pharmacol. 2014 Jan;37(1):228-33. doi: 10.1016/j.etap.2013.11.027. Epub 2013 Dec 7.

[46] Gutowska I1, Baranowska-Bosiacka I, Siwiec E, Szczuko M, Kolasa A, Kondarewicz A, Rybicka M, Dunaj-Stanczyk M, Wiernicki I, Chlubek D, Stachowska E. Lead enhances fluoride influence on apoptosis processes in liver cell line HepG2. Toxicol Ind Health. 2013 Nov 5. [Epub ahead of print]

decreased significantly after addition of Pb in a dose-dependent manner. When used together, fluoride inhibited the decreasing effect of Pb on cell proliferation."

Wen (2012)[47] The aim of this study was to explore the association of parathyroid hormone (PTH) gene Bst BI polymorphism, calciotropic hormone levels, and dental fluorosis of children. A case-control study was conducted in two counties (Kaifeng and Tongxu) in Henan Province, China in 2005-2006. Two hundred and twenty-five children were recruited and divided into three groups including dental fluorosis group (DFG), non-dental fluorosis group (NDFG) from high fluoride areas, and control group (CG). Urine fluoride content was determined using fluoride ion selective electrode; PTH Bst BI were genotyped using PCR-RFLP; osteocalcin (OC) and calcitonin (CT) levels in serum were detected using radioimmunoassay. Genotype distributions were BB 85.3% (58/68), Bb 14.7% (10/68) for DFG; BB 77.6% (52/67), Bb 22.4% (15/67) for NDFG; and BB 73.3% (66/90), Bb 27.7% (24/90) for CG. No significant difference of Bst BI genotypes was observed among three groups ($P > 0.05$). Serum OC and urine fluoride of children were both significantly higher in DFG and NDFG than in CG ($P < 0.05$, respectively), while a similar situation was not observed between DFG and NDFG in high fluoride areas ($P > 0.05$). Serum OC level of children with BB genotype was significantly higher compared to those with Bb genotype in high fluoride areas ($P < 0.05$). However, no significant difference of serum CT or calcium (Ca) was observed. In conclusion, there is no correlation between dental fluorosis and PTH Bst BI polymorphism. Serum OC might be a more sensitive biomarker for detecting early stages of dental fluorosis, and further studies are needed.

The parathyroid gland produces parathyroid hormone (PTH). PTH regulates the amount of calcium in our bones and blood supply. When the calcium level in blood starts to fall, PTH triggers the breakdown of bone tissue as a means of transferring the body's stored supply of calcium into the blood supply. When the parathyroid produces too much PTH a condition known as hyperparathyroidism develops. Hyperparathyroidism has been found to occur as a secondary effect of the fluoride-induced bone disease skeletal fluorosis, and may help to explain some of the bone effects encountered in fluorosis.

When calcium is removed from the bones (osteoclastic activity) the fluoride in the bones increases blood fluoride concentrations.

Gupta et al. (2001)[48] and Suketa (2002) show again that in cases of fluorosis there is hyperparathyroidism, as seen in elevated parathyroid hormone (PTH) levels.

Acevedo (1996)[49] Chardin (1998)[50] When thyroid and parathyroid glands are removed in subjects, same mineral effects occur as can be observed in dental fluorosis patients.

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[47] Wen S1, Li A, Cui L, Huang Q, Chen H, Guo X, Luo Y, Hao Q, Hou J, Ba Y., The relationship of PTH Bst BI polymorphism, calciotropic hormone levels, and dental fluorosis of children in China., Biol Trace Elem Res. 2012 Jun;147(1-3):84-90. doi: 10.1007/s12011-011-9313-5. Epub 2012 Jan 5.

[48] Gupta SK, Khan TI, Gupta RC, Gupta AB, Gupta KC, Jain P, Gupta A - "Compensatory hyperparathyroidism following high fluoride ingestion - a clinico - biochemical correlation" Indian Pediatr 38(2):139-46 (2001)

Stamp (1990)[51]

> "1. To determine the relationships between parathyroid hormone activity and long-term sodium fluoride therapy in osteoporosis . . . .
> 2. Cross-sectional data showed a fourfold mean increase in biologically active parathyroid hormone on fluoride treatment . . . .
> 3. Fluoride-treated patients were then analysed in two groups according to the level of biologically active parathyroid hormone. . . .
> 4. Results show that long-term fluoride and calcium therapy increase biologically active parathyroid hormone in osteoporosis and that excessive parathyroid hormone activity may account for certain features of the refractory state."

Chen (1988)[52] "Fluoride ion (F-) alone or in conjunction with aluminum (Al3+) has been shown to stimulate the activity of guanine nucleotide-binding proteins (G proteins) in cell membrane preparations from a variety of cell types and in intact hepatic cells. Several studies have indicated that G proteins are involved in the regulation of parathyroid hormone (PTH) secretion. Intracellular second messengers which modulate PTH secretion (e.g., cAMP) have also been found to be regulated by G proteins. We have, therefore, employed F- as a probe to investigate the possible role of G proteins in the modulation of PTH release and the intracellular second messengers that have been implicated in the control of PTH secretion. F- produces a dose-dependent inhibition of PTH release with a maximal inhibitory effect (67%) at 5 mM. F- exerts its inhibitory effect within 5 min and the degree of suppression of PTH secretion gradually increases over 1 hr. F- (5 mM) inhibits PTH secretion at 0.5 mM Ca2+ to the level observed with 2 mM Ca2+ alone; moreover, the effects of F- and high Ca2+ are not additive. . . . We conclude that F- is a potent inhibitor of PTH secretion."

Mertz (1987)[53] "Fluorine is known to bind calcium in the body, causing ionic calcium to decrease; this, in turn, causes secondary hyperparathyroidism."

However, more recent investigations have revealed that a new mechanism of action: hyperparathyroidism is caused by chronically elevated TSH levels. (Fluoride is **the** TSH clone]. Elevated TSH levels are usually seen in hypothyroidism, and therefore explain

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[49] Acevedo AC, Chardin H, Staub JF, Septier D, Goldberg M - "Morphological study of amelogenesis in the rat lower incisor after thyro-parathyroidectomy, parathyroidectomy and thyroidectomy." Cell Tissue Res 283(1):151-7 (1996)

[50] Chardin H, Acevedo AC, Risnes S - "Scanning electron microscopy and energy-dispersive X-ray analysis of defects in mature rat incisor enamel after thyroparathyroidectomy." Arch Oral Biol 43(4):317-27 (1998)

[51] Stamp TC1, Saphier PW, Loveridge N, Kelsey CR, Goldstein AJ, Katakity M, Jenkins MV, Rose GA. Fluoride therapy and parathyroid hormone activity in osteoporosis. Clin Sci (Lond). 1990 Sep;79(3):233-8.

[52] Chen CJ1, Anast CS, Brown EM. Effects of fluoride on parathyroid hormone secretion and intracellular second messengers in bovine parathyroid cells. J Bone Miner Res. 1988 Jun;3(3):279-88.

[53] [Trace Elements in Human and Animal Nutrition - Fifth Edition, Edited by Walter Mertz, U. S. Dept. of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, Maryland, p. 375 (1987)

why hyperparathyroidism is so closely associated with hypothyroidism (Paloyan et al,1997).[54]

Hyperparathyroidism is ten times more frequent in thyroid patients than expected in a general medical population and is especially prevalent in patients with **goiter** (Stoffer, 1982).

Roy (1962) “These experiments may be interpreted to show that the effect of NaF is to reduce the solubility of the apatite complex and thus to lower the basic level of equilibrium of calcium between fluid and solid phases. To compensate for this decreased level, the glands of the intact animals are required to increase secretion with an ultimate increase in osteoclast proliferation.”[55]

[54] Paloyan Walker R, Kazuko E, Gopalsami C, Bassali J, Lawrence AM, Paloyan E - "Hyperparathyroidism associated with a chronic hypothyroid state" Laryngoscope 107(7):903-9 (1997)

[55] Roy V. Talmage, S.B. Doty The effect of sodium fluoride on parathyroid function in the rat as studied by peritoneal lavage General and Comparative Endocrinology Volume 2, Issue 5, October 1962, Pages 473–479

C. PANCREAS:

The pancreas produces a hormone called insulin which regulates the uptake of glucose from the bloodstream. Fluoride increases the levels of glucose in the blood. Vinals provides a review and background of the mechanism which fluoride acts on the insulin receptors and is moved to the top of the list of studies to provide a foundation.

Vinals (1993)[56] "Fluoride is a nucleophilic reagent which has been reported to inhibit a variety of different enzymes such as esterases, asymmetrical hydrolases and phosphatases. In this report, we demonstrate that fluoride inhibits tyrosine kinase activity of insulin receptors partially purified from rat skeletal muscle and human placenta. Fluoride inhibited in a similar dose-dependent manner both β-subunit autophosphorylation and tyrosine kinase activity for exogenous substrates. This inhibitory effect of fluoride was not due to the formation of complexes with aluminium and took place in the absence of modifications of insulin-binding properties of the insulin receptor. Fluoride did not compete with the binding site for ATP or Mn2+. Fluoride also inhibited the autophosphorylation and tyrosinekinase activity of receptors for insulin-like growth factor I from human placenta. Addition of fluoride to the pre-phosphorylated insulin receptor produced a slow (time range of minutes) inhibition of receptor kinase activity. Furthermore, fluoride inhibited tyrosine kinase activity in the absence of changes in the phosphorylation of pre-phosphorylated insulin receptors, and the sensitivity to fluoride was similar to the sensitivity of the unphosphorylated insulin receptor. The effect of fluoride on tyrosine kinase activity was markedly decreased when insulin receptors were pre-incubated with the copolymer of glutamate/tyrosine. Prior exposure of receptors to free tyrosine or phosphotyrosine also prevented inhibitory effect of fluoride. However, the protective effect of erosion or phosphotyrosine was maximal at low concentrations, suggesting the interaction of these compounds with the receptor itself rather than with fluoride. These data suggest: (i) that fluoride interacts directly and slowly with the insulin receptor, which causes inhibition of its phosphotransferase activity; (ii) that the binding site of fluoride is not structurally modified by receptor phosphorylation; and (iii) based on the fact that fluoride inhibits phosphotransferase activity in the absence of alterations in the binding of ATP, Mn2+ or insulin, we speculate that fluoride binding might affect the transfer of phosphate from ATP to the tyrosine residues of the β-subunite of the insulin receptor and to the tyrosine residues of exogenous substrates.
"The insulin receptor is a disulphide-linked herotetrameric membrane glycoprotein consisting of two alph (M 135000) and two transmembrane beta (M 95000) subunits (Massague et al., 1981); Massage and Czech, 1982; Ullrich et al, 1985; Ebina et al., 1985). The alpha subunits are entirely extracellular and participate in insulin binding, whereas the beta-subunits contain extracellular , transmembrane and intracellular domains. . . . The tyrosine kinase activity of the insulin receptor appears to be essential for certain cellular responses to insulin. Thus anti-insulin-receptor antibodies, which inhibit the kinase activity of the insulin receptors, also block the ability of cells to

[56] VINALS F, TESTAR X, PALACIN M and ZORZANO A. Inhibitory effect of fluoride on insulin receptor autophosphorylation and tyrosinekinase activity, "Biochem.J.(1993)291,615-622(PrintedinGreatBritain) 615

respond to insulin (Morgan et al., 1986; Morgan and Roth, 1987). In addition, the microinjection of insulin receptors in *Xenopus* oocytes causes an increase in the phosphorylation of ribosomal S6 subunit, which is further increased by prior receptor activation, due to insulin-receptor autophosphorylation (Maller et al., 1986). Studies with receptors mutated at the ATP-binding site (Chou et al., 1987; Ebina et al., 1987; McClain et al., 1987) or at tyrosine residues 1162 and 1163 (Ellis et al., 1986; Decant et al., 1988) have also led to the conclusion that that tyrosine phosphotransferase function of the insulin receptor is an absolute requirement for the hormone to activate the receptor signaling function in cells.

"Based on the pivotal role of insulin-receptor kinase activity on insulin action, the catalytic properties of the insulin-receptor kinase require thorough characterization. In studies initially designed to investigate the interaction between regulatory G-proteins and insulin receptors, we substantiated a potent inhibitory effect of fluoride on insulin-receptor kinase activity. On the basis of this finding and the fact that the use of fluoride , a potent nucleophilic reagent (Edwards and Pearson, 1962), has yielded useful information on the kinetics of a variety of enzymes (Layne and Najjar, 1975; Bunick and Kashket, 1982; Nilsson and Branden, 1982), we have characterized the inhibitory effect of fluoride on insulin-receptor autophosphorylation and receptor kinase for exogenous substrates."

(A few references primarily in author alphabetical order are provided here. I have not read each article and only a few quotes which were handy, are included here.)

Adebayo 2012[57] "We conclude that fluoride exerts biochemical effect on **lipid peroxidation** and antioxidant enzymes of both PU and well-fed rats. This effect varied widely between the liver and the pancreas but it seems that the liver is more sensitive to the toxic assault of fluoride than the pancreas especially in PU rats."

Agalakova (2012)[58] "The molecular mechanisms underlying fluoride toxicity are different by nature. Fluoride is able to stimulate G-proteins with subsequent activation of downstream signal transduction pathways such as PKA-, PKC-, PI3-kinase-, Ca2+-, and MAPK-dependent systems. G-protein-independent routes include tyrosine phosphorylation and protein phosphatase inhibition. Along with other toxic effects, fluoride was shown to induce oxidative stress leading to excessive generation of ROS, lipid peroxidation, decrease in the GSH/GSSH ratio, and alterations in activities of antioxidant enzymes, as well as to inhibit glycolysis thus causing the depletion of cellular ATP and disturbances in cellular metabolism. Fluoride triggers the disruption of mitochondria outer membrane and release of cytochrome c into cytosol, what activates caspases-9 and -3 (intrinsic) apoptotic

[57] Olusegun Lateef Adebayo and Gbenga Adebola Adenuga, 2012. Biochemical Changes in the Liver and the Pancreas of Well-fed and Protein Undernourished Rats Following Fluoride Administration. *Asian Journal of Applied Sciences, 5: 215-223.*

[58] Natalia Ivanovna Agalakova and Gennadii Petrovich Gusev. Molecular Mechanisms of Cytotoxicity and Apoptosis Induced by Inorganic Fluoride, ISRN Cell Biology, Volume 2012 (2012), Article ID 403835, 16 pages http://dx.doi.org/10.5402/2012/403835

pathway. Extrinsic (death receptor) Fas/FasL-caspase-8 and -3 pathway was also described to be implicated in fluoride-induced apoptosis. Fluoride decreases the ratio of antiapoptotic/proapoptotic Bcl-2 family proteins and upregulates the expression of p53 protein. Finally, fluoride changes the expression profile of apoptosis-related genes and causes endoplasmic reticulum stress leading to inhibition of protein synthesis.

Banu P et al. Toxicity of fluoride to diabetic rats. Fluoride 1997 30(1) 43-50.

Birkner E, et al. Influence of sodium fluoride and caffeine on the concentration of fluoride ions, glucose, and urea in blood serum and activity of protein metabolism enzymes in rat liver. Bill Trace Elem Res. 2006 112(2) 169-74.

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NRC (2006) page 214. "OTHER ENDOCRINE ORGANS "The effects of fluoride exposure have been examined for several other endocrine organs, including the adrenals, the pancreas, and the pituitary (for details, see Appendix E, Tables E-16 and E-17). Effects observed in animals include changes in organ weight, morphological changes in tissues, increased mitotic activity, decreased concentrations of pituitary hormones, depressed glucose utilization, elevated serum glucose, and elevated insulin-like growth factor-1 (IGF-1). Effects reported in humans include "endocrine disturbances," impaired glucose tolerance, and elevated concentrations of pituitary hormones. Studies of the effects of fluoride on glucose metabolism and in diabetic animals are discussed below; information on other effects is extremely limited.

Pan (2015)[62] "Two-dimensional gel electrophoresis (2-DE) was used to detect fluoride-induced alterations in the proteome of the rat hippocampus. Male Sprague-Dawley rats (n=30) were subjected to treatments three weeks after weaning. Animals of the first group were injected intraperitoneally (i.p.) with aqueous NaF (20 mg/kg/body weight/day), the second group, injected with physiological saline, served as the control. After 30 days, the body weight of the fluoride-treated rats was lower than that of the control, and F- levels in serum were higher than in the control. The hippocampus was subjected to proteomic analysis, and the fluoride-treated group was found to contain 19 up-regulated and eight down-regulated proteins. The proteins, identified by mass-spectroscopic analysis of their fragments obtained after digestion, were found to be involved in amino acid biosynthesis, the insulin signaling pathway and various other crucial functions. Our results also provide useful information on the mechanism of the reduction of the learning ability and memory induced by F."

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D. PINEAL GLAND:

In the seventeenth century, Descartes called the pineal gland the seat of the soul, the connection between the intellect and the body.[64] The pineal gland is about the size of a grain of rice (5mm X 8 mm) the only unpaired midline brain structure. It is located just below the brain in the quadrigeminal cistern and part of the epithalamus. It produces the hormone melatonin which regulates the body's circadian rhythm as well as the onset of puberty (See: Schlesinger ER, Overton DE, Chase HC, Cantwell KT (1956). Newburgh-Kingston caries-fluorine study X111. Pediatric findings after ten years. J Amer Dent Assoc 52: 296-306).

The NRC (2006) review of the literature to that date should be carefully considered and is quoted here.

"Pineal Gland Calcification

> "The pineal gland is a calcifying tissue; in humans, calcified concretions can be found at any age, although the likelihood increases with age (Vígh et al. 1998; Akano and Bickler 2003) and may be associated with menopause (Sandyk et al. 1992). The occurrence of pineal calcifications varies among different populations and nations (Vígh et al. 1998), possibly in association with the degree of industrialization (Akano and Bickler 2003), rates of breast cancer (Cohen et al. 1978), and high circannual light intensity near the equator (Vígh et al. 1998). Osteoporosis might be associated with fewer concretions (Vígh et al. 1998).
>
> "Melatonin secretion is well correlated with the amount of uncalcified pineal tissue (Kunz et al. 1999) but not with the size of pineal calcification (Vígh et al. 1998; Kunz et al. 1999). An increase in calcification of the pineal gland in humans probably represents a decrease in the number of functioning pinealocytes and a corresponding decrease in the individual's ability to produce melatonin (Kunz et al. 1999). The degree of calcification, relative to the size of an individual's pineal gland, has been suggested as a marker of the individual's decreased capability to produce melatonin (Kunz et al. 1999).
>
> "As with other calcifying tissues, the pineal gland can accumulate fluoride (Luke 1997, 2001). Fluoride has been shown to be present in the pineal glands of older people (14-875 mg of fluoride per kg of gland in persons aged 72-100 years), with the fluoride concentrations being positively related to the calcium concentrations in the pineal gland, but not to the bone fluoride, suggesting that pineal fluoride is not necessarily a function of cumulative fluoride exposure of the individual (Luke 1997, 2001). Fluoride has not been measured in the pineal glands of children or young adults, nor has there been any investigation of the relationship between pineal fluoride concentrations and either recent or cumulative fluoride intakes.

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[64] Descartes and the Pineal Gland (Stanford Encyclopedia of Philosophy)
Descartes R. "The Passions of the Soul" excerpted from "Philosophy of the Mind," Chalmers, D. New York: Oxford University Press, Inc.; 2002. ISBN 978-0-19-514581-6

“In Vitro Studies

“Few studies have examined the effects of fluoride on pineal function. NaF (2.5-20 mM, or fluoride at 47.5-380 mg/L) produces markedly increased adenylyl cyclase activity (up to four times control activity) of rat pineal homogenates in vitro (Weiss 1969a,b), as it does in other tissues (Weiss 1969a); ATPase activity in the homogenates was inhibited by up to 50% (Weiss 1969a). Potassium fluoride (7-10 mM, or fluoride at 133-190 mg/L) has been used experimentally to increase adenylyl cyclase activity in rat pineal glands in vitro (Zatz 1977, 1979).

“Animal Studies

“Details of the effect of fluoride on pineal function are presented in Appendix E, Table E- 15. Luke (1997) examined melatonin production as a function of age and time of day in Mongolian gerbils (Meriones unguiculatus). On an absolute basis, melatonin production by the low-fluoride group was constant at ages 7-28 weeks, with no difference between males and females. Relative to body weight, melatonin output declined progressively with age until adulthood (by 11.5 weeks in females and 16 weeks in males). In contrast, prepubescent gerbils fed the high-fluoride diet had significantly lower pineal melatonin production than prepubescent gerbils fed the low-fluoride diet. Relative to body weight, the normal higher rate of melatonin production in sexually immature gerbils did not occur.

“Sexual maturation in females occurred earlier in the high-fluoride animals (Luke 1997); males had increases in melatonin production relative to body weight between 11.5 and 16 weeks (when a decrease normally would occur), and testicular weight at 16 weeks (but not at 9 or 28 weeks) was significantly lower in high-fluoride than in low-fluoride animals. The circadian rhythm of melatonin production was altered in the high-fluoride animals at 11.5 weeks but not at 16 weeks. In high-fluoride females at 11.5 weeks, the nocturnal peak (relative to body weight) occurred earlier than in the low-fluoride animals; also, the peak value was lower (but not significantly lower) in the high-fluoride animals. In males, a substantial reduction ($P < 0.00001$) in the nocturnal peak (relative to body weight) was observed in the high-fluoride animals.

“Human Studies

“Although no studies are available that specifically address the effect of fluoride exposure on pineal function or melatonin production in humans, two studies have examined the age of onset of menstruation (age of menarche) in girls in fluoridated areas (Schlesinger et al. 1956; Farkas et al. 1983; for details, see Appendix E, Table

E-15) ; the earlier study was discussed by Luke (1997) as part of the basis for her research. No comparable information on sexual maturation in boys is available.”

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Both Schlesinger et al. (1956) and Farkas et al. (1983) referred to tables of the distribution of ages at the time of first menstruation, but, in fact, both studies provided only frequencies by age (presumably at the time of study, in either 1-year or 0.5-year increments) of girls having achieved menarche by the stated age. Farkas et al. (1983) specifically indicated use of the probit method for ascertainment of the median age at menarche; the data provided by Schlesinger et al. (1956) appear to correspond to that method, but they do not specifically mention it. The probit (or status quo) method appears to be routinely used to estimate the median (or other percentiles of) age at menarche, sometimes in conjunction with an estimated mean age at menarche based on recall data (e.g., Wu et al. 2002; Anderson et al. 2003; Chumlea et al. 2003; Padez and Rocha 2003). According to Grumbach and Styne (2002), “The method of ascertainment of the age of menarche is of importance. Contemporaneous recordings are performed with the probit method of asking, ‘yes’ or ‘no,’ are you menstruating? These may be incorrect because of social pressures of the culture and socioeconomic group considered. Recalled ages of menarche are used in other studies and considered to be accurate within 1 year (in 90% of cases) during the teenage years and in older women, too.”

“In girls examined approximately 10 years after the onset of fluoridation (1.2 mg/L, in 1945) in Newburgh, New York, the average age at menarche was 12 years, versus 12 years 5 months among girls in unfluoridated Kingston (Schlesinger et al. 1956). The authors stated that this difference was not statistically significant. Note that those girls who reached menarche during the time period of the study had not been exposed to fluoride over their entire lives, and some had been exposed perhaps for only a few years before menarche (they would have been 8-9 years old at the time fluoridation was started). Those girls in Newburgh who had been exposed to fluoridated water since birth (or before birth) had not yet reached menarche by the time of the study.

“A later study in Hungary (Farkas et al. 1983) reported no difference in the menarcheal age of girls in a town with “optimal” fluoride concentration (1.09 mg/L in Kunszentmárton, median menarcheal age 12.779 years) and a similar control town (0.17 mg/L in Kiskunmajsa; median menarcheal age 12.79 years). This study shows postmenarcheal girls present at younger ages in the higher fluoride town than in the low-fluoride town, although the reported median ages were the same (Farkas et al. 1983).

“Discussion (Pineal Function)

“Whether fluoride exposure causes decreased nocturnal melatonin production or altered circadian rhythm of melatonin production in humans has not been investigated. As described above, fluoride is likely to cause decreased melatonin production and to have other effects on normal pineal function, which in turn could contribute to a variety of effects in humans. Actual effects in any individual depend on age, sex, and probably other factors, although at present the mechanisms are not fully understood.”

Luke (2001)[65] "By old age, the pineal gland has readily accumulated F and its F/Ca ratio is higher than bone. . . The pineal gland is a mineralizing tissue. . . The concretions are composed of hydroxyapatitie (HA). . . calcium is distributed throughout the pinealocytes: in the mitochondria, golgi apparatus, cytoplasm, and nucleus. Fluoride does not accumulate in the brain. Of all tissues, brain has the lowest fluoride concentrations. It is generally agreed that the blood-brain barrier restricts the passage of fluoride into the central nervous system. The human pineal gland is outside the blood-brain barrier. . . . pinealocytes have free access to fluoride in the bloodstream. This fact, coupled with the presence of HA, suggest that the pineal gland may sequester fluoride from the bloodstream." See Luke's graph below.

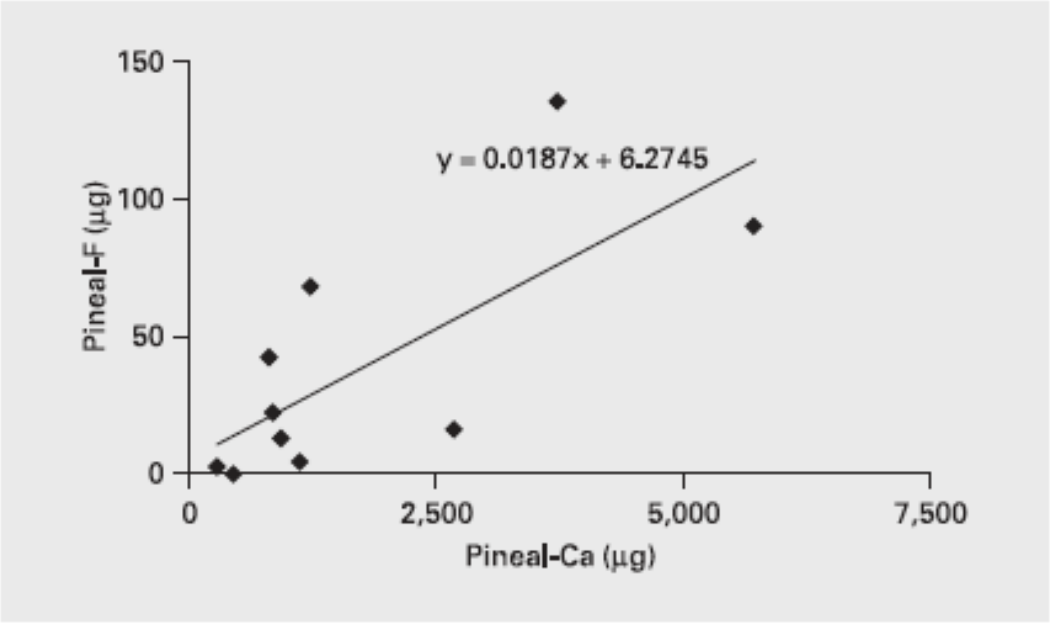

Fig. 1. The relationship between the calcium and fluoride contents of ten aged human pineal glands.

The pineal gland is bathed in cerebrospinal fluid but is not isolated by the blood brain barrier and is second only to the kidneys in blood profusion. (After the blood brain barrier is formed, the barrier mitigates fluoride transmission, but not for the pineal gland who's blood source is outside the blood brain barrier.) Innervation is sympathetic, parasympathetic, from the otic ganglia and trigeminal ganglion with nerve fibers containing the neuropeptide PACAP.

The pineal gland consists mainly of two types of pinealocytes, like photoreceptors, and

[65] Luke, J., Fluoride deposition in the human Pineal Gland. Caries Research | 2001; 35(2):125-128 | School of Biological Sciences, University of Surrey, Guildford, UK.

decline by way of apoptosis as the age of the organism increases.[66] High concentrations of fluoride and other toxins cause apoptosis. Type 1 cells are high in mitochondria and convert the amino acid tryptophan to serotonin then N-acetyl-serotonin and then to melatonin. Type 2 contain vacuoles, melatonin and are thought to act like endocrine and neuronal cells.[67]

Pinealocytes contain synaptic ribbons in children and adults but not human fetuses. Synaptic ribbons are important in neurotransmitter release.[68]

One of the difficulties in studying the pineal gland is the significant difference between rodents and higher vertebrates with rodent pineal gland lacking pineal gland neurons.

Although the effects of high concentrations of fluoride remain poorly understood, animal experiments have found that high doses of fluoride had a reduced melatonin production and an earlier onset of puberty.

The abundant melatonin levels in children are believed to inhibit sexual development which maybe a mechanism for early puberty with increased fluoride exposure.

> "Studies on rodents suggest that the pineal gland may influence the actions of recreational drugs, such as cocaine,[69] and antidepressants, such as fluoxetine (Prozac),[70] and its hormone melatonin can protect against neurodegeneration."[71]
>
> It is only a matter of time before researchers more clearly elucidate whether fluoride's effect is a contributing or causative factor for calcification and apoptosis of the pineal gland and the resulting decrease in melatonin production, early puberty and insomnia.

Kalisinska (2014) "Fluoride concentration in the pineal gland was significantly greater than in the bone and the brain of the duck."[72]

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[68] Spiwoks-Becker, I., C. Maus, S. Dieck, A. Fejtová, L. Engel, T. Wolloscheck, U. Wolfrum, L. Vollrath, and R. Spessert (2008). "Active Zone Proteins Are Dynamically Associated with Synaptic Ribbons in Rat Pinealocytes". *Cell and Tissue Research* **333** (2): 185–95. doi:10.1007/s00441-008-0627-3. PMC 2757586. PMID 18523806.

[69] Uz T, Akhisaroglu M, Ahmed R, Manev H (2003). "The pineal gland is critical for circadian Period1 expression in the striatum and for circadian cocaine sensitization in mice". *Neuropsychopharmacology* **28** (12): 2117–23. doi:10.1038/sj.npp.1300254. PMID 12865893

[70] Uz T, Dimitrijevic N, Akhisaroglu M, Imbesi M, Kurtuncu M, Manev H (2004). "The pineal gland and anxiogenic-like action of fluoxetine in mice". *Neuroreport* **15** (4): 691–4. doi:10.1097/00001756-200403220-00023. PMID 15094477.

[71] Manev H, Uz T, Kharlamov A, Joo J (1996). "Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats". *FASEB J* **10** (13): 1546–51. PMID 8940301.

[72] Kalisinska E1, Bosiacka-Baranowska I, Lanocha N, Kosik-Bogacka D, Krolaczyk K, Wilk A, Kavetska K, Budis H, Gutowska I, Chlubek D. Fluoride concentrations in the pineal gland, brain and bone of goosander (Mergus

merganser) and its prey in Odra River estuary in Poland. Environ Geochem Health. 2014 Dec;36(6):1063-77. doi: 10.1007/s10653-014-9615-6. Epub 2014 Apr 18.

E. Adrenal Gland

Schetinina 1997)[73] The activity of carboxypeptidase (CP) H, the enzyme taking part in neuropeptide formation, and activity of recently described phenylmethylsulfonyl fluoride (PMSF)--inhibiting CP in males and females of white mongrel rats were studied. Minor differences between the CPH activities in brain regions were found in hippocampus. PMSF-inhibited activity of carboxypeptidase was significantly higher in females than in males in pituitary gland, adrenal gland, olfactory bulbus, optic and auditory bills, cerebellum, hippocampus, striatum, cerebral hemispheres and spleen. The CPH activity was 5-fold higher in ovaries than in testicles. PMSF-inhibited CP activity in testicles was 3.7-fold lower than in ovaries. Possible participation of basic CP in determination of sexual differences of some neuropeptide level and protein catabolism is studied.

Juska (1995)[74] A mathematical model relating the activity of adenylate cyclase (AC) with concentrations of stimulators, equilibrium dissociation constants, specific activity and efficacies of AC depending on the states of its binding sites has been developed and used for analysis of the data on activation of AC of bovine adrenal cortex plasma membranes presented in (De Foresta et al. (1987) FEBS Lett. 216, 107-112). Equilibrium dissociation constants. chi h and chi l, corresponding to high- and low-affinity forskolin-binding sites were estimated to be 0.37 and 17 microM: these constants characterize forskolin's potency more adequately than does ED50, the concentration eliciting half-asymptotic activity of AC. Corticotropin does not affect the affinity of AC for forskolin whereas fluoride increases this affinity, thus augmenting forskolin's potency. . . .”

Cannon (1994)[75] “Guanine nucleotide binding proteins (G proteins) act as signal transducers between membrane receptors and ion channels. In the present study, the whole-cell arrangement of the patch clamp technique was used to examine the effect of G proteins on K+ channels in cultured bovine adrenal chromaffin cells . . . . Treatment of the chromaffin cells with fluoride decreased nicotine-evoked secretion of catecholamines in a concentration-dependent manner. . . .”

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73 Shchetinina NVVernigora ANGengin MTAuthor information [Basic carboxypeptidase activity in rats of both sexes]. [Article in Russian] Ukr Biokhim Zh (1978). 1997 May-Jun;69(3):115-8.

74 Juska A, de Foresta B .Analysis of effects of corticotropin, forskolin and fluoride on activity of adenylate cyclase of bovine adrenal cortex. Biochim Biophys Acta. 1995 Jun 14;1236(2):289-98

75 Cannon SD[1], Wilson SP, Walsh KB. A G protein-activated K+ current in bovine adrenal chromaffin cells: possible regulatory role in exocytosis. Mol Pharmacol. 1994 Jan;45(1):109-16

Vitale (1993)[76] **“**The use of non-hydrolyzable analogues of GTP in permeabilized secretory cells suggests that guanine nucleotide-binding regulatory proteins (G proteins) may be involved in regulated exocytosis. . . . These results suggest that the secretory machinery in chromaffin cells can be blocked by activating a G(o) protein. Consistent with this finding, two other known activators of heterotrimeric G proteins, aluminum fluoride and benzalkonium chloride, inhibited calcium-evoked catecholamine secretion in streptolysin O-permeabilized chromaffin cells. We conclude that an inhibitory G(o) protein, possibly located on the membrane of secretory granules, is involved in the final stages of exocytosis in chromaffin cells.”

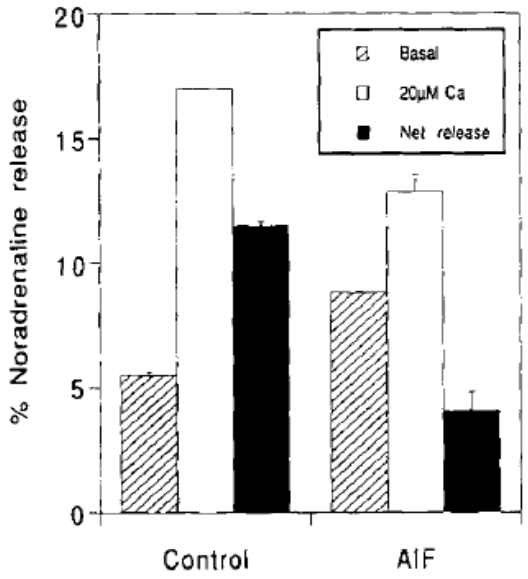

FIG. 6. **Effect of AlF_4^- on secretion from SLO-permeabilized chromaffin cells.** SLO-permeabilized cells were preincubated for 10 min in calcium-free KG medium in the presence (*AlF*) or absence (*Control*) of 20 mM NaF and 50 μM $AlCl_3$. Cells were then stimulated for 10 min with KG medium containing 20 μM free calcium (*open columns*). The basal release was estimated in calcium-free KG medium (*scratched columns*) and subtracted to obtain the net noradrenaline release (*closed columns*). AlF_4^- inhibited calcium-dependent noradrenaline release in chromaffin cells.

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76 Vitale N[1], Mukai H, Rouot B, Thiersé D, Aunis D, Bader MF. J Biol Chem. Exocytosis in chromaffin cells. Possible involvement of the heterotrimeric GTP-binding protein G(o).
1993 Jul 15;268(20):14715-23.

Ito (1991)[77] We have reported recently that prostaglandin E2 (PGE2) stimulated phosphoinositide metabolism in bovine adrenal chromaffin cells and that PGE2 and ouabain, an inhibitor of Na+, K(+)-ATPase, synergistically induced a gradual secretion of catecholamines from the cells. Here we examined the involvement of a GTP-binding protein(s) in PGE receptor-induced responses by using NaF. In the presence of Ca2+ in the medium, NaF stimulated the formation of all three inositol phosphates, i.e., inositol monophosphate, bisphosphate, and trisphosphate, linearly over 30 min in a dose-dependent manner (15-30 mM). This effect on phosphoinositide metabolism was accompanied by an increase in cytosolic free Ca2+. NaF also induced catecholamine release from chromaffin cells, and the dependency of stimulation of the release on NaF concentration was well correlated with those of NaF-enhanced inositol phosphate formation and increase in cytosolic free Ca2+. Although the effect of NaF on PGE2-induced catecholamine release in the presence of ouabain was additive at concentrations below 20 mM, there was no additive effect at 25 mM NaF. Furthermore, the time course of catecholamine release stimulated by 20 mM NaF in the presence of ouabain was quite similar to that by 1 microM PGE2, and both stimulations were markedly inhibited by amiloride, with half-maximal inhibition at 10 microM. Pretreatment of the cells with pertussis toxin did not prevent, but rather enhanced, PGE2-induced catecholamine release over the range of concentrations examined. These results demonstrate that NaF mimics the effect of PGE2 on catecholamine release from chromaffin cells and suggest that PGE2-evoked catecholamine release may be mediated by the stimulation of phosphoinositide metabolism through a putative GTP-binding protein insensitive to pertussis toxin.

De Foresta (1987)[78] “The diterpene forskolin maximally stimulated bovine adrenal cortex adenylate cyclase activity 9-fold with a concentration producing half-maximum effect (ED50) of about 4 microM. The effects of forskolin and the fully active corticotropin fragment ACTH (I 24) were additive over nearly the whole range of concentration of both effectors, indicating separate and independent mechanisms of action. By contrast, 10 mM NaF blocked forskolin action in the nanomolar range of the diterpene concentration, while it allowed a partial stimulation by forskolin in the micromolar range. NaF thus reveals a heterogeneity of forskolin action in the adrenal cortex plasma membranes. Moreover, our data suggest that ACTH and NaF activation effects, both mediated by the stimulatory regulatory protein Gs, proceed through different mechanisms.”

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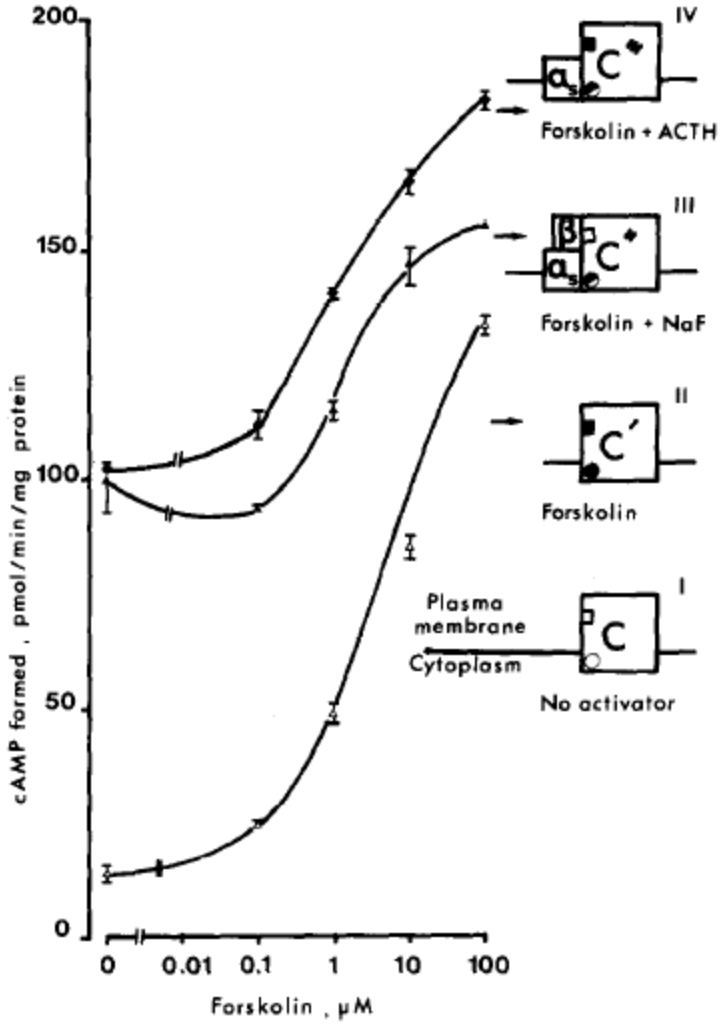
77 Ito S[1], Negishi M, Mochizuki-Oda N, Yokohama H, Hayaishi O., Sodium fluoride mimics the effect of prostaglandin E2 on catecholamine release from bovine adrenal chromaffin cells. J Neurochem. 1991 Jan;56(1):44-51.

78 de Foresta B, Rogard M, Gallay J., Adenylate cyclase of bovine adrenal cortex plasma membranes. Divergence between corticotropin and fluoride combined effects with forskolin. FEBS Lett. 1987 May 25;216(1):107-12.



Suketa (1985)[79] "Changes in adrenal function as a possible mechanism for elevated serum glucose by a single large dose of fluoride."

Wolff (1970)[80] "Chlorpromazine (3 x 10(-4)M) prevents the stimulation of adenyl cyclase activity in thyroid membranes produced by thyrotropin and prostaglandin, ACTH stimulation of adenyl cyclase in adrenal tissue, and glucagon- and epinephrine-stimulation of adenyl cyclase activity in liver. Baseline activity is unaffected. Parathyroid hormone stimulation of kidney preparations was not inhibited under these conditions. At chlorpromazine concentrations >3 x 10(-4)M F(-)-stimulated cyclase activity of thyroid and adrenal tissue was increased. Other phenothiazines, trifluoperazine, and prochlorperazine, have similar effects on thyrotropin and F(-)-

[79] Suketa Y, Asao Y, Kanamoto Y, et al. "Changes in adrenal function as a possible mechanism for elevated serum glucose by a single large dose of fluoride." To Appl Pharm. 1985. 80 199-205.

[80] Wolff J, Jones ABInhibition of hormone-sensitive adenyl cyclase by phenothiazines. Proc Natl Acad Sci U S A. 1970 Feb;65(2):454-9

stimulated cyclase activity of thyroid. Na(+)- K(+)-dependent ATPase of thyroid is also inhibited by chlorpromazine. Since thymol causes a similar dissociation of hormone- and F(-)-stimulated adenyl cyclase, it is concluded that the surface properties of these agents best account for their effects on adenyl cyclase.”

F. GONADS

Ovaries: The first study is by Yin (2015), and a significant portion is presented here because it illustrates the risks better and confirms earlier studies with depth.

Yin (2015)[81] "Reproductive toxicity has been an exciting topic of research in reproductive biology in recent years. Soluble fluoride salts are toxic at high concentrations; their reproductive toxicity was assessed in this study by administering different fluoride salt concentrations to mice. Continuous feeding for five weeks resulted in damage to the histological architecture of ovaries. The expression of genes, including Dazl, Stra8, Nobox, Sohlh1, and ZP3 gene, associated with oocyte formation were much lower in the experimental group as compared with the control group. The number of in vitro fertilization of mature oocytes were also much lower in the experimental group as compared with control. Moreover, the fertility of female mice, as assessed by mating with normal male mice, was also lower in experimental compared with control groups. The expression of the oocyte-specific genes: Bmp15, Gdf9, H1oo, and ZP2, which are involved in oocyte growth and the induction of the acrosome reaction, decreased with the fluoride administration. DNA methylation and histone acetylation (H3K18ac and H3K9ac) are indispensable for germline development and genomic imprinting in mammals, and fluoride administration resulted in reduced levels of H3K9ac and H3K18ac in the experimental group as compared with the control group, as detected by immunostaining. Our results indicate that the administration of high concentrations of fluoride to female mice significantly reduced the number of mature oocytes and hampered their development and fertilization. Thus, this study lays a foundation for future studies on fluoride-induced reproductive disorders in women.

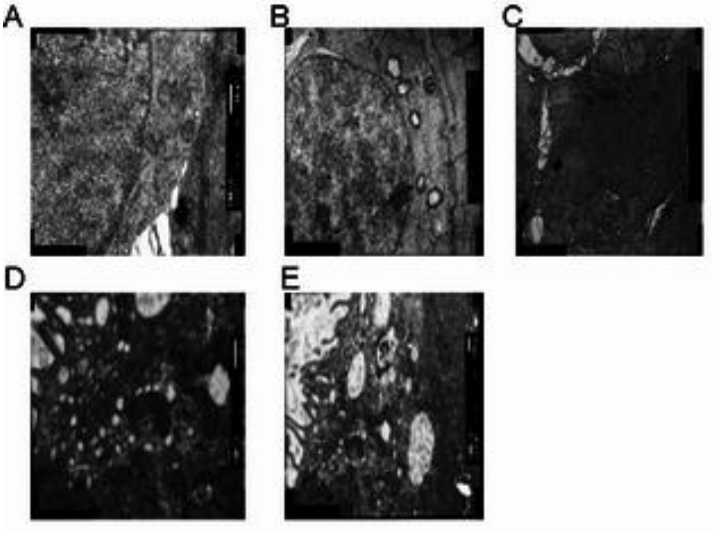


Fig 1. Effect of fluoride administration on ultrastructural features of ovary. (A-E): Ovaries were removed from female mice and ultrathin sections were cut. The histological architecture of ovaries from the control group (A, administered 0 mg/L NaF) and experimental (B-E; administered 50, 100, 150, and 200 mg/L NaF, respectively) groups was examined by transmission electron microscopy.

[81] Yin S[1], Song C[1], Wu H[1], Chen X[1], Zhang Y[1]. Adverse Effects of High Concentrations of Fluoride on Characteristics of the Ovary and Mature Oocyte of Mouse. PLoS One. 2015 Jun 8;10(6):e0129594. doi: 10.1371/journal.pone.0129594. eCollection 2015.

Effect of fluoride administration on expression of germline-specific genes in the ovary

RNA was isolated from ovaries of mice from the control and experimental groups, and the expression of potential germline-specific genes, particularly Dazl, Stra8, Sohlh1, Nobox, and Zp3, was analysed by RT-PCR. As observed in Fig 2A–2E, the expression of these genes was lower in the experimental groups (administered 50, 100, 150, or 200mg/L NaF) compared with the control group (administered 0mg/L NaF). Increase in fluoride concentration resulted in the decreased expressions of these genes, particularly Nobox, which was rarely detected in the experimental groups (Fig 2D).

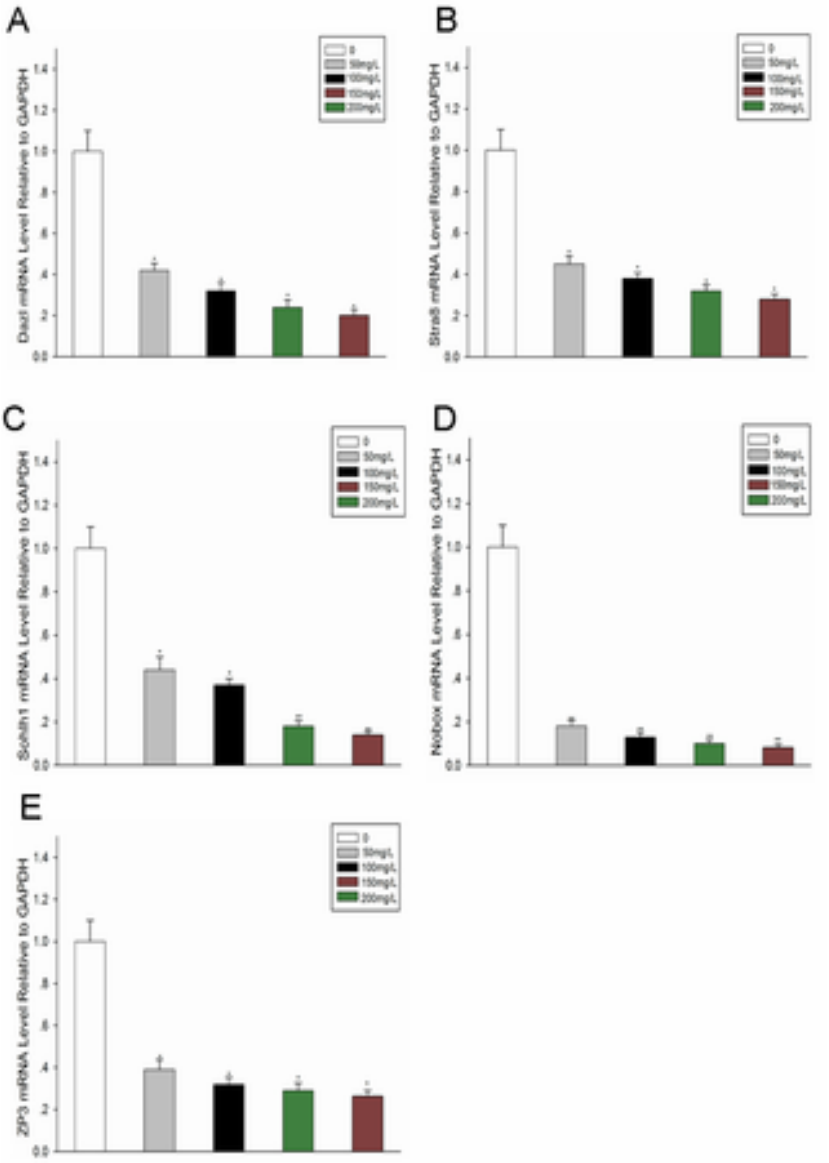


Fig 2. Effect of fluoride administration on expression of germline-specific genes in the ovary.

(A-E): mRNA was harvested from ovaries of mice from the control and experimental groups. qPCR was performed for assessing the relative expression levels of germline-specific genes (A: Dazl, B: Stra8, C: Sohlh1, D: Nobox, and E: Zp3) in the ovary. All data are presented as the mean ± SD and are derived from three independent experiments. *P<0.05; **P<0.01.

Effect of fluoride administration on the formation and in vitro/in vivo fertilization of mature oocytes

The effect of high concentrations of fluoride on the formation and in vitro fertilization of mature oocytes was investigated; furthermore, the fertility of female mice exposed to fluorides was examined by mating with normal male mice. Superovulation was achieved by the administration of 10 IU pregnant mare serum gonadotropin and 10 IU human chorionic gonadotropin before mating or harvesting of mature oocytes from the oviduct ampullae, as detailed in Materials and Methods. Fig 3A shows that the number of mature oocytes per ovary was significantly lower in the experimental groups (administered 50, 100, 150, or 200 mg/L NaF) compared with the control group (administered 0 mg/L NaF). This result is also reflected in the lower fertility of fluoride-administered female mice, as assessed by mating with normal male mice (Fig 3B), and in the lower efficiency of in vitro fertilization for the experimental groups compared with the control group (Fig 3C).

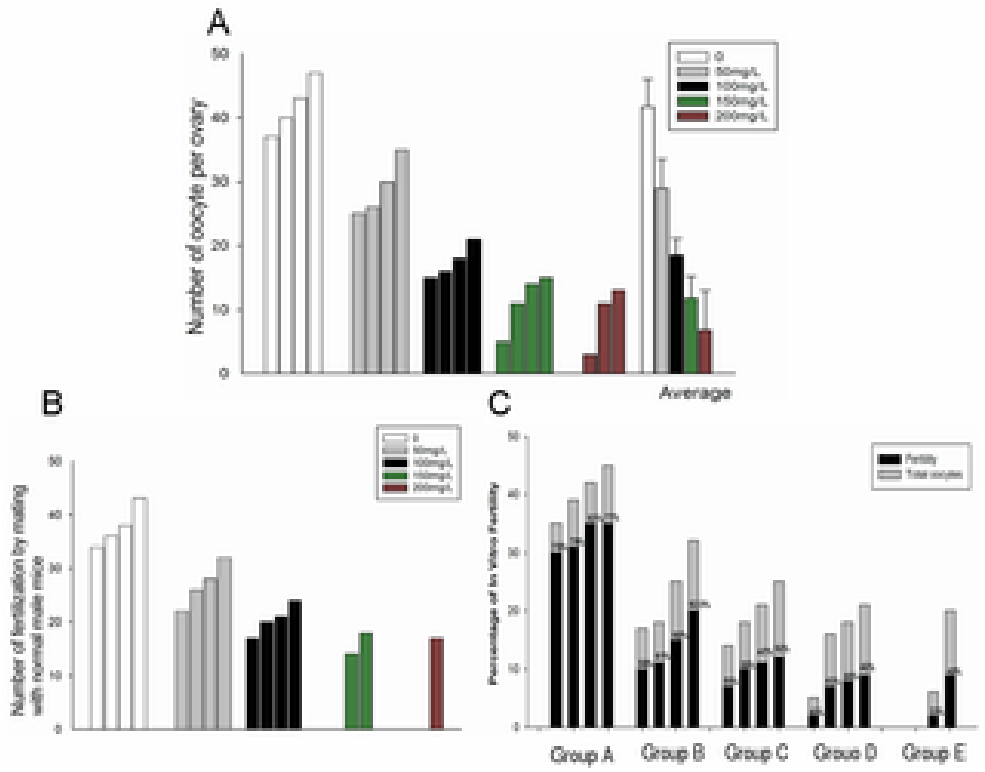


Fig 3. Effect of fluoride administration on formation and in vitro/in vivo fertilization of mature oocytes.

Mature oocytes were released from oviduct ampullae of superovulated mice ~14–16 h following the administration of human chorionic gonadotropin, and the number of the mature oocytes in the ovaries (A) and the efficiency of in vitro fertilization (C)were estimated. Mice from the control and experimental groups were mated with normal male mice following the administration of human chorionic gonadotropin for detecting the in vivo fertilization efficiency (B). (Data are presented as mean ± SD, with four mice (n = 4) per group).

Effect of fluoride administration on the expression of oocyte-specific genes

The results mentioned above indicate that the number and fertilization of mature oocytes are affected by high concentrations of fluoride. Therefore, the expression of oocyte-specific genes was evaluated by RT-PCR following the direct synthesis of cDNA from mature oocytes, as detailed in Materials and Methods. Four oocyte-specific genes, Bmp15, Gdf-9, Zp2, and H1oo, were focused on in this study because of their crucial functions. Expression of all these genes was found to be lower in the experimental groups compared with the control group, with negative association observed between the expression of these genes and fluoride concentration (Fig 4A–4D).

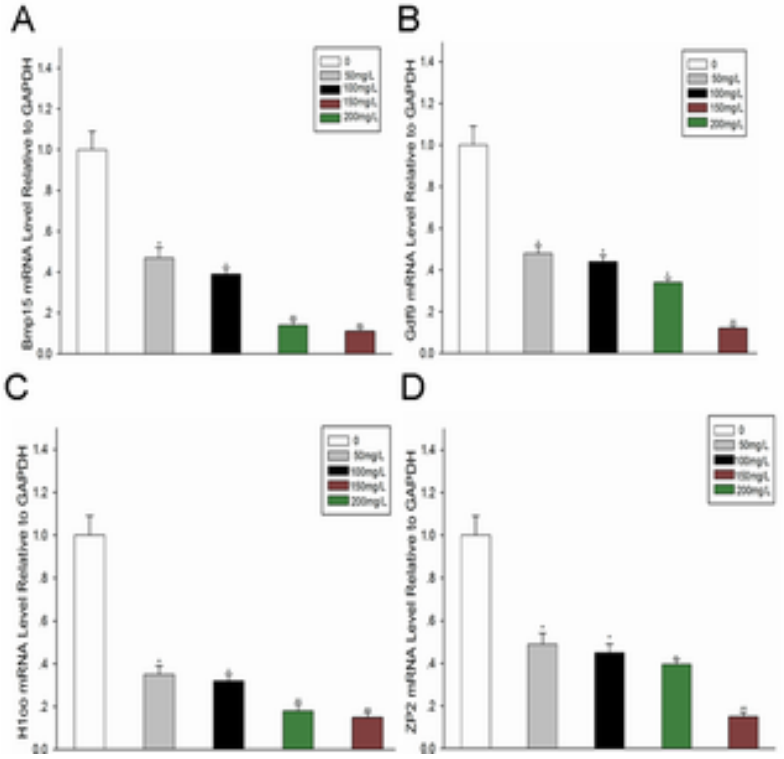


Fig 4. Effect of fluoride administration on the expression of oocyte-specific genes.

(A-D): mRNA was harvested from oocytes of mice from the control and experimental groups. RT-PCR was performed for assessing the relative expression levels of oocyte-specific genes (A: Bmp15, B: Gdf9, C: H1oo, and D: Zp2) in the oocytes. All data are presented as the mean ± SD and are derived from three independent experiments. *P<0.05; **P<0.01.

Effect of fluoride administration on DNA methylation and histone acetylation in mature oocytes

Immunostaining was performed to assess the effect of fluoride administration on global DNA methylation and histone acetylation (notably, H3K18ac and H3K9ac) in mature oocytes. As seen in Fig 5A, significant differences were not observed in 5-methylcytosine levels between the experimental (administered various fluoride

concentrations) and control groups. In contrast, lower levels of H3K18ac and of the H3K9ac were observed in the experimental groups (Fig 5B and 5C). (Not included)

Fig 5. Effect of fluoride administration on DNA methylation and histone acetylation in mature oocytes.
Mature oocytes were released from the oviduct ampullae of superovulated mice ~14–16 h following the administration of human chorionic gonadotropin. Immunofluorescence was performed for the detection of levels of 5-methylcytosine (A), H3K9ac (B), and H3K18ac (C). Each sample was stained with anti-5-methylcytosine (green), anti-H3K9ac (green), or anti- H3K18ac (green) antibodies and counterstained with DAPI (blue) to allow DNA visualization. Samples were visualized at (original magnification × 200) for exposure time of 200 ms (anti-5-methylcytosine, anti-H3K9ac and anti-H3K18ac).

Discussion

Fluorides are well recognized as pollutants, with a great deal of research focused on the environmental hazard that they cause [22, 23]. While the effects of fluoride exposure on fertility are known, its exact effect on the production of mature oocytes in mammalian ovaries remains to be investigated. The objective of this study is to explicitly assess the adverse effects of high concentrations of fluoride on the characteristics of mouse ovary and mature oocyte.

The consumption of large quantities of fluoride administration resulted in obvious damage to the histological architecture of mouse ovaries, as reported previously [14, 24]. Further, the effect of fluoride administration on the expression of germline-specific genes was investigated. Previous studies have reported the association between expression of particular ovary-specific genes and oocyte formation. Dazl, expressed during embryonic development in the female gonads of mice well before the onset of meiosis, functions in the first phase of gametogenesis during the differentiation, proliferation and maintenance of primordial germ cells and their substitutes [25]; Stra8 is required for meiotic progression in the mouse ovary, previous studies demonstrated that meiosis is a sex-specific event where germ cells undergo cellular differentiation to form oocytes or spermatozoa, with abnormal gene expression during meiosis leading to aberrant gamete formation, which is often a major cause of infertility in both males and females [26]; Nobox deficiency has been shown to disrupt early folliculogenesis and expression of oocyte-specific genes [27]; Sohlh1 is a transcription factors of the bHLH family and is specifically expressed in germ cells; it plays a role in oocyte differention, in female, such that Sohlh1 ablation causes oocyte loss in the neonatal ovary [28]; Zp3 plays an important role in the development of mouse zona pellucida, which is critical for fertilization [29]. This study revealed that the expression of these genes was much lower in the experimental groups compare with the control group and showed an inverse association with the concentration of fluoride adminstratered. The changes in histological architecture and expression of germline-specific genes in the ovary are likely to affect the formation and fertilization of mature oocytes. The effect of high

concentrations of fluoride on the formation of mature oocytes was investigated by inducing superovulation followed by collection of mature oocytes; moreover, in vitro fertilization and in vivo fertilization following mating with normal male mice were also assessed. The results obtained showed that increase in fluoride concentration resulted in lower yield of mature oocytes as well as lower efficiency of in vivo and in vitro fertilization in the experimental groups compared with the control group, which is in agreement with the observed expression of germline-specific genes, as detailed above.

The expression of the following oocyte-specific genes was also assessed following fluoride administration: Bmp15, which is involved in oocyte maturation and follicular development; Gdf-9, which regulates the oocyte growth and function of oocytes as well as growth and differention of granulose cell; zp2, which mediates species-specific sperm binding, induces acrosome reaction, and prevents post fertilization polyspermy; and H1oo, whose expression is restricted to the growing/maturing oocyte and to the zygote [30]. The expression of these oocyte-specific genes was decreased upon fluoride administration, which is expected to disrupt the normal maturation of oocyte.

The important role played by histone acetylation and DNA methylation in oogenesis is widely accepted. Previous studies have shown that occurrence of 5-methylcytosine in mammals genomes is crucial for normal mammalian development, while histone acetylation is associated with a transcriptionally active state and allows access of transfactor to DNA sequence. Abnormal epigenetic modification is expected to be detrimental to offspring as a consequence of DNA damage [31]. Therefore, the levels of global DNA methylation, and the active histone marks H3K9ac and H3K18ac were assessed in mature oocytes following the administration of fluoride to mice. The results revealed the absence of significant differences in the level of 5-methylcytosine between the experimental and control groups. However, the levels of H3K9ac and H3K18ac were lower in the experimental compared with the control groups and decreased with increase in fluoride concentration. Such abnormal epigenetic modification is likely to be particularly detrimental to offspring.

Behavioral differences were also observed in mice belonging to various experimental groups. Mice belonging to the experimental group D (administered 150 mg/L NaF) were observed to be thinner compared with the other groups, while the mice of group E (administered 200 mg/L NaF) consumed a much greater quantity of water; moreover, the mice of groups C, D, and E (administered 100, 150, and 200 mg/L NaF, respectively) displayed a tendency to closely approach one another. This is attributable to the neurotoxicity and behavioral changes caused upon fluoride consumption in animals [32, 33].

Taken together, this study suggests that the administration of high concentrations of fluoride to female mice not only results in ovarian damage but also significantly reduces the number and the fertilization potential of mature oocytes by reducing the expression of genes that play an important role in the normal development and maturation of oocytes. The results obtained in this study could thus be employed for statistical analysis of the association between exposure to high concentrations of fluoride and reproductive disorders in women.”

Geng (2014)[82] The toxicity of sodium fluoride (NaF) to female fertility is currently recognized; however, the mechanisms are unclear. Previously, we reported a reduction in successful pregnancy rates, ovarian atrophy and dysfunction following exposure to NaF. The purpose of this study was to elucidate the underlying molecular mechanisms. Female Sprague-Dawley rats (10 rats/group) received 100 or 200mg/L NaF in their drinking water for 6 months or were assigned to an untreated control group. Apoptotic indices and oxidative stress indicators in blood and ovarian tissue were analyzed following sacrifice. The results confirmed the NaF-induced ovarian apoptosis, with concomitant activation of oxidative stress. Further investigations in ovarian granular cells showed that exposure to NaF activated extracellular regulated protein kinase (ERK) and c-Jun NH2 kinase (JNK), disrupting the ERK and JNK signaling pathways, while p38 and PI3K remained unchanged. These data demonstrated that oxidative stress may play a key role in NaF-induced ovarian dysfunction by activating the apoptotic ERK and JNK signaling pathways.

Zhou (Feb 2013)[83] "The aim of this study was to investigate the effects of sodium fluoride (NaF) on female reproductive function and examine the morphology of the ovaries and uteri of rats exposed to NaF. . . . These results suggest that female reproductive function is inhibited by NaF and that exposure to NaF causes ovarian and uterine structural damage. NaF may thus significantly reduce the fertility of female rats."

Zhou (Sept 2013),[84] "Recognition of the harmful effects of sodium fluoride (NaF) on human reproduction is increasing, especially as it relates to female reproduction. However, the mechanism by which NaF interferes with female reproduction is unclear. The aims of the present study were to investigate the effects of fluoride exposure on female fertility and to elucidate the mechanisms underlying these effects. . . . These results suggest that the reproductive hormone reduction and the abnormalities of related receptor proteins expression are important factors underlying the decreased fertility observed in female rats that have been exposed to NaF."

Johanna (2013)[85] The effects of oral administration of sodium fluoride (NaF) and/or arsenic trioxide (As(2)O(3)) (5 mg and 0.5 mg/kg body weight, respectively) for 30 days were investigated on free radical induced toxicity in the mouse ovary. The

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[82] Geng Y[1], Qiu Y[2], Liu X[3], Chen X[4], Ding Y[5], Liu S[6], Zhao Y[7], Gao R[8], Wang Y[9], He J[10]. Sodium fluoride activates ERK and JNK via induction of oxidative stress to promote apoptosis and impairs ovarian function in rats. J Hazard Mater 2014 May 15;272:75-82. doi: 10.1016/j.jhazmat.2014.03.011. Epub 2014 Mar 18.

[83] Zhou Y[1], Zhang H, He J, Chen X, Ding Y, Wang Y, Liu X. Effects of sodium fluoride on reproductive function in female rats. Food Chem Toxicol. 2013 Jun;56:297-303. doi: 10.1016/j.fct.2013.02.026. Epub 2013 Feb 28.

[84] Zhou Y[1], Qiu Y, He J, Chen X, Ding Y, Wang Y, Liu X. The toxicity mechanism of sodium fluoride on fertility in female rats. Food Chem Toxicol. 2013 Dec;62:566-72. doi: 10.1016/j.fct.2013.09.023. Epub 2013 Sep 23.

[85] Jhala DD[1], Chinoy NJ, Rao MV. Mitigating effects of some antidotes on fluoride and arsenic induced free radical toxicity in mice ovary. Food Chem Toxicol. 2008 Mar;46(3):1138-42. doi: 10.1016/j.fct.2007.11.009. Epub 2007 Nov 23.

reversibility of the induced effects after withdrawal of NaF+As(2)O(3) treatment and by administration of antioxidant vitamins (C, E) and calcium alone as well as in combination were also studied. The combined treatment of NaF and As(2)O(3) impaired significantly ($p<0.001$) the production of free radical scavengers such as glutathione and ascorbic acid as well as antioxidant enzymes, namely, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (Cat), thereby increasing ovarian lipid peroxides (LPO) which might have rendered the ovary susceptible to injury. The withdrawal of the combined (NaF and As(2)O(3) for 30 days) treatment caused partial recovery in the ovary, which was more pronounced ($p<0.001$) by treatment with vitamin C, calcium, or vitamin E alone and in combination. Hence the induced toxicity was transient and reversible.

Hou (2013) "To explore the influence of water fluoride exposure on reproductive hormones in female. Cross-sectional study was conducted in seven villages of a county in Henan province by using simple random sampling including high fluoride area, defluoridation project area and control area on April, 2011 based on the preliminary study results of fluoride concentration in drinking water. Women who were born and growth or lived in the village at least 5 years and aged 18-48 years old were recruited using cluster sampling. They were divided into high fluoride group (HFG, 116 subjects), defluoridation project group (DFPG, 132 subjects) and control group (CG, 227 subjects) in accordance with the above areas. All subjects accepted questionnaire and physical checkup. . . Fluoride exposure may influence reproductive hormones in female, especially in ovulatory and luteal phase of menstrual cycle."[86]

The Oxford Journals (2006)[87] is many pages in length and a good source for review of the ovary and developing follicle. In part, they report:

"Ovarian follicle development is a complex process that begins with the establishment of what is thought to be a finite pool of primordial follicles and culminates in either the atretic degradation of the follicle or the release of a mature oocyte for fertilization. This review highlights the many advances made in understanding these events using transgenic mouse models. Specifically, this review describes the ovarian phenotypes of mice with genetic mutations that affect ovarian differentiation, primordial follicle formation, follicular growth, atresia, ovulation and corpus luteum (CL) formation. In addition, this review describes the phenotypes of mice with mutations in a variety of genes, which affect the hormones that regulate folliculogenesis. Because studies using transgenic animals have revealed a variety of reproductive abnormalities that resemble many reproductive disorders in women, it is likely that studies using transgenic mouse models will impact our understanding of ovarian function and fertility in women."

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[86] Hou JX1, Yang YJ, Gong B, Li SH, Ding Z, Wen SB, Li SQ, Cheng XM, Cui LX, Ba Y. [The influence of high fluoride exposure in drinking water on endocrine hormone in female]. [Article in Chinese] Zhonghua Yu Fang Yi Xue Za Zhi. 2013 Feb;47(2):142-6.

[87] Ovarian follicle development and transgenic mouse models, Hum. Reprod. Update Oxford Journals (September/October 2006) 12 (5): 537-555. doi: 10.1093/humupd/dml022 First published online: May 25, 2006 Update (September/October 2006) 12 (5): 537-555. doi: 10.1093/humupd/dml022

Stan (1994)[88] “A review of fluoride toxicity showed decreased fertility in most animal species studied. The current study was to see whether fluoride would also affect human birth rates. A U.S. database of drinking water systems was used to identify index counties with water systems reporting fluoride levels of at least 3 ppm. These and adjacent counties were grouped in 30 regions spread over 9 states. For each county, two conceptionally different exposure measures were defined, and the annual total fertility rate (TFR) for women in the age range 10–49 yr was calculated for the period 1970–1988. For each region separately, the annual TFR was regressed on the fluoride measure and sociodemographic covariables. Most regions showed an association of decreasing TFR with increasing fluoride levels. Meta-ana/ysis of the region-specific results confirmed that the combined result was a negative TFR/fluoride association with a consensus combined p value of .0002-.0004, depending on the analytical scenario. There is no evidence that this outcome resulted from selection bias, inaccurate data, or improper analytical methods. However, the study is one that used population means rather than data on individual women. Whether or not the fluoride effect on the fertility rate found at the county level also applies to individual women remains to be investigated.”

[88] Stan C. Freni., Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. Journal of Toxicology and Environmental Health, 1994, Volume 42, Issue 1, pages 109-121

TESTES:

Han (2015)[89] "Numerous studies have shown that fluoride exposure adversely affected the male reproductive function, while the molecular mechanism is not clear. The present study was to investigate the effects of fluoride exposure (60days) on the expressions of reproductive related genes, serum sex hormone levels and structures of the hypothalamus-pituitary-testicular axis (HPTA), which plays a vital role in regulating the spermatogenesis in male mice. In this study, 48 male mice were administrated with 0, 25, 50, and 100mg/L NaF through drinking water. Results showed that the malformation ratio of sperm was significantly increased ($P<0.05$). At transcriptional level, the expression levels of follicle-stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), inhibin alpha (INHα), inhibin beta-B (INHβB), and sex hormone binding globulin (SHBG) mRNA in testis were significantly decreased ($P<0.05$). Moreover, histological lesions in testis and ultrastructural alterations in hypothalamus, pituitary and testis were obvious. However, the same fluoride exposure did not lead to significant changes of related mRNA expressions in hypothalamus and pituitary ($P>0.05$). Also, there were no marked changes in serum hormones. Taken together, we conclude that the mechanism of HPTA dysfunction is mainly elucidated through affecting testes, and its effect on hypothalamus and pituitary was secondary at exposure for 60days."

Hamza (2015)[90] "Sodium fluoride (NaF) intoxication is associated with oxidative stress and altered antioxidant defense mechanism. The present study was carried out to evaluate the potential protective role of blackberry and quercetin (Q) against NaF-induced oxidative stress and histological changes in liver, kidney, testis and brain tissues of rats. . . .RESULTS AND CONCLUSIONS: NaF caused an elevation in lipid peroxidation level paralleled with significant decline in glutathione peroxidase, glutathione reductase, glutathione S-transferase, superoxide dismutase and catalase activities as well as the total antioxidant activity in liver, kidney, testes and brain. Some histopathological changes were detected in all tested tissues of the NaF treated group. Q and BBJ had successfully maintained normal histological architecture and mitigated the induction of oxidative stress caused by NaF. Q effectively reduced the elevation in thiobarbituric acid reactive substances level and restored the activities of antioxidant enzymes in liver, kidney, testis and brain. Less histopathological changes were observed in Q+NaF and BBJ+NaF treated groups. As a result, BBJ and Q significantly reduced NaF-induced oxidative and histological changes in rats. In the combination of BBJ and Q against NaF toxicity, the effects were more severe than from separate exposure, thus indicating that these flavonoids exhibited synergistic effects on all antioxidant and histological parameters."

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[89] Han H1, Sun Z1, Luo G2, Wang C3, Wei R1, Wang J4., Fluoride exposure changed the structure and the expressions of reproductive related genes in the hypothalamus-pituitary-testicular axis of male mice. Chemosphere. 2015 Sep;135:297-303.

[90] Hamza RZ, El-Shenawy NS, Ismail HA. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. J Basic Clin Physiol Pharmacol. 2015 May;26(3):237-51.

Song (2014)[91] '"The biological effects of fluoride on human health are often extensive, either beneficial or detrimental. Among the various effects of fluoride exposure in different organs, the reproductive tract is particularly susceptible to disruption by fluoride at a sufficient concentration. It has attracted much attention to the effect of sodium fluoride on male fertility, gestational female, and offspring. Herein, we applied a widespread natural compound sodium fluoride (NaF) and investigated the effects of acute NaF exposure on Leydig cells, including their proliferation, apoptosis, and signal pathway changes. Our results demonstrated that high dosage of NaF could inhibit cell proliferation by stress-induced apoptosis, which was confirmed by cellular and molecular evidences. We found that fluoride exposure affected the expression levels of stress response factors, signal transduction components, and apoptosis-related proteins, including caspase-3/caspase-9, B-cell lymphoma 2 (Bcl-2), and Bax. This study suggests that the complex effects of fluoride on Leydig cells are closely related to its dosage."

Geng (2014) "The toxicity of sodium fluoride (NaF) to female fertility is currently recognized; however, the mechanisms are unclear. Previously, we reported a reduction in successful pregnancy rates, ovarian atrophy and dysfunction following exposure to NaF. The purpose of this study was to elucidate the underlying molecular mechanisms. . . The results confirmed the NaF-induced ovarian apoptosis, with concomitant activation of oxidative stress. Further investigations in ovarian granular cells showed that exposure to NaF activated extracellular regulated protein kinase (ERK) and c-Jun NH2 kinase (JNK), disrupting the ERK and JNK signaling pathways, while p38 and PI3K remained unchanged. These data demonstrated that oxidative stress may play a key role in NaF-induced ovarian dysfunction by activating the apoptotic ERK and JNK signaling pathways."[92]

Wang (2014) "Sodium fluoride (NaF) has been found to interfere with the reproductive system of animals. However, the cellular mechanisms underlying the reproductive toxicity of fluoride are unclear. The present study aims to define a possible mechanism of NaF-induced reproductive toxicity with respect to mineral, oxidative stress and c-Fos expression and the role of aluminum (Al) in intervening the toxic effect of NaF on rat testes. . . The present study suggested that NaF could decrease the contents of Ca, Fe and Mg and enhance oxidative stress leading to c-Fos overexpression, and some deleterious effects were more prominent at lower NaF intake. Furthermore, Al within the research concentration could minimize reproductive toxicity caused by fluoride."[93]

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[91] Song Gh[1], Wang RL, Chen ZY, Zhang B, Wang HL, Liu ML, Gao JP, Yan XY. Toxic effects of sodium fluoride on cell proliferation and apoptosis of Leydig cells from young mice. J Physiol Biochem. 2014 Sep;70(3):761-8. doi: 10.1007/s13105-014-0344-1. Epub 2014 Jul 30.

[92] Geng Y1, Qiu Y2, Liu X3, Chen X4, Ding Y5, Liu S6, Zhao Y7, Gao R8, Wang Y9, He J10. Sodium fluoride activates ERK and JNK via induction of oxidative stress to promote apoptosis and impairs ovarian function in rats. J Hazard Mater. 2014 May 15;272:75-82. doi: 10.1016/j.jhazmat.2014.03.011. Epub 2014 Mar 18.

[93] Wang J1, Zhang H, Xu F, Xu F, Zhang K, Zhang Y. The antagonism of aluminum against fluoride-induced oxidative stress and c-Fos overexpression in rat testes. Toxicol Mech Methods. 2014 Feb;24(2):136-41. doi: 10.3109/15376516.2013.869779. Epub 2013 Dec 16.

Yang (2014)[94] “Investigated the effects of N-acetylcysteine (NAC) on endoplasmic reticulum stress of sertoli cells induced by sodium fluoride (NaF). METHODS: Rat sertoli cells were exposed to various concentration of (0, 6, 12, 24 μg/ml) sodium fluoride with or without 2 mmol/L NAC for 24 hours. The cell viability was evaluated using trypan blue exclusion test. Intracellular reactive oxygen species (ROS) was measured using the fluorescent probe DCFH-DA. Western blot was used to test the expression of GRP78, PERK and CHOP. RESULTS: It was found that treatment with NAC (2 mmol/L) restored the reduced cell viability and excessive oxidative stress (P < 0.01). Moreover, fluoride exposure upregulated the expression of GRP7 8, PERK and CHOP protein (P <0. 01). NAC was also found to suppress the levels of GRP78, PERK and CHOP expression in NaF-treated cells (p<0.01). CONCLUSION: Endoplasmic reticulum stress signaling pathways were activated by ROS, and NAC attenuate endoplasmic reticulum stress through inhibiting the levels of ROS in NaF-treated sertoli cells.”

Zhang (2013)[95] “Long-term excessive fluoride intake is known to be toxic and can damage a variety of organs and tissues in the human body. However, the molecular mechanisms underlying fluoride-induced male reproductive toxicity are not well understood. In this study, we used a rat model to simulate the situations of human exposure and aimed to evaluate the roles of endoplasmic reticulum (ER) stress and inflammatory response in fluoride-induced testicular injury. Sprague-Dawley rats were administered with sodium fluoride (NaF) at 25, 50 and 100mg/L via drinking water from pre-pregnancy to gestation, birth and finally to post-puberty. And then the testes of male offspring were studied at 8weeks of age. Our results demonstrated that fluoride treatment increased MDA accumulation, decreased SOD activity, and enhanced germ cell apoptosis. In addition, fluoride elevated mRNA and protein levels of glucose-regulated protein 78 (GRP78), inositol requiring ER-to-nucleus signal kinase 1 (IRE1), and C/EBP homologous protein (CHOP), indicating activation of ER stress signaling. Furthermore, fluoride also induced testicular inflammation, as manifested by gene up-regulation of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), in a nuclear factor-κB (NF-κB)-dependent manner. These were associated with marked histopathological lesions including injury of spermatogonia, decrease of spermatocytes and absence of elongated spermatids, as well as severe ultrastructural abnormalities in testes. Taken together, our results provide compelling evidence that ER stress and inflammation would be novel and significant mechanisms responsible for fluoride-induced disturbance of spermatogenesis and germ cell loss in addition to oxidative stress.”

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[94]Yang Y, Huang H, Feng D, Liu W, Cheng X, Ba Y, Cui L.[Effects. of N-acetylcysteine on fluoride-induced endoplasmic reticulum stress in sertoli cells]. [Article in Chinese] Wei Sheng Yan Jiu. 2014 Sep;43(5):805-8, 813.

[95]Zhang S[1], Jiang C, Liu H, Guan Z, Zeng Q, Zhang C, Lei R, Xia T, Gao H, Yang L, Chen Y, Wu X, Zhang X, Cui Y, Yu L, Wang Z, Wang A. Fluoride-elicited developmental testicular toxicity in rats: roles of endoplasmic reticulum stress and inflammatory response. Toxicol Appl Pharmacol. 2013 Sep 1;271(2):206-15. doi: 10.1016/j.taap.2013.04.033. Epub 2013 May 22.

Deng (2013) “To discuss the significance of calcineurin (CaN) and nuclear factor of active T cells 1 (NFATc1) in the damage mechanism of the testis of rats with chronic fluorosis. . . The changes in the signaling pathway of expression of CaN may be involved in the injury mechanism of testis tissues of rats with chronic fluorosis.”[96]

Dimcevici (2013) “It has been revealed that excessive fluoride intake on long-term is associated with toxic effects and can damage a variety of organs and tissues in the human body, including the male reproductive system. . . The results indicate that natrium fluoride administered in different doses, even at homeopathic dose or at allopathic-homeopathic dose, determined vacuolar dystrophy of epididymal epithelial cells, vacuolar dystrophy of linear seminal cells and necrosis.”[97]

Xiao (2011)[98] “The rat fluorosis models were successfully established. The fluoride content in testis was significantly increased in all the fluorosis groups(P<0.01). Testicular structures were damaged in all of fluoride groups. The TNOS, iNOS activities, and MDA content of each fluoride group were significantly higher than that of the control group on day 120 and 180 (P<0.05 or 0.01). The TNOS, iNOS activities, and MDA content significantly increased in a dose dependent manner (P<0.05 or 0.01). The SOD activities significantly decreased in all the fluoride groups (P<0.05 or 0.01). **CONCLUSIONS:** Endemic fluoride poisoning caused by coal burning can cause disorders in the oxidative system and antioxidative system in rat testis. The oxidative stress may play an important role in the fluorides induced reproductive toxicity in male rats.

Hao (2010)[99] **OBJECTIVE:** To study of endocrine disturbing effect of fluoride on human hypothalamus-hypophysis-testis axis hormones. **METHODS:** Sunying County, Kaifeng City was selected as polluted district which the fluoride in drinking water was 3.89 mg/L, and Shenlilou county was selected as control district which the fluoride was less than 1.0 mg/L. 150 individual lived there more than 5 years were selected randomly. And investigated by medical examination, then blood and urine sample were collected, and the serum level of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), testosterone (T) and estradiol (E2) were measured by RIA method, and the urine level of fluoride were measured. Other than that, the concentration of fluoride in the water, food, soil and air were detected by the

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[96] Deng CN1, Yu YN2, Xie Y1, Zhao LN1. [Expression of calcineurin and nuclear factor of activated T cells 1 in testis of rats with chronic fluorosis]. [Article in Chinese] Zhonghua Yu Fang Yi Xue Za Zhi. 2013 Dec;47(12):1142-7.

[97] Dimcevici Poesina N1, Bălălău C, Bârcă M, Ion I, Baconi D, Baston C, Băran Poesina V. Testicular histopathological changes following sodium fluoride administration in mice. Rom J Morphol Embryol. 2013;54(4):1019-24.

[98] Xiao YH[1], Sun F, Li CB, Shi JQ, Gu J, Xie C, Guan ZZ, Yu YN.[Effect of endemic fluoride poisoning caused by coal burning on the oxidative stress in rat testis]. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2011 Aug;33(4):357-61. doi: 10.3881/j.issn.1000-503X.2011.04.002 [Article in Chinese]

[99] Hao P[1], Ma X, Cheng X, Ba Y, Zhu J, Cui L.[Effect of fluoride on human hypothalamus-hypophysis-testis axis hormones]. Wei Sheng Yan Jiu. 2010 Jan;39(1):53-5. [Article in Chinese]

standard methods. **RESULTS:** The concentrations of fluoride in the water, food and soil of the fluoride polluted district were significantly higher than those of control district ($P < 0.05$), and the concentration fluoride in the air of two district were not found. There was no significant difference of serum level of GnRH between fluoride polluted district and control district ($P > 0.05$). The serum level of LH in men of fluoride polluted district was significantly higher than that of control group ($P < 0.05$), and the serum level of T in men of fluoride polluted district was significantly less than that of control group ($P < 0.05$). There was no significant difference of serum level of LH between fluoride polluted district and control district ($P > 0.05$), and the serum level of T in women of fluoride polluted district was significantly higher than that of control group ($P < 0.05$). There was no significant difference of serum level of E2 between fluoride polluted district and control district ($P > 0.05$). **CONCLUSION:** Fluoride could effect hormone levels of each layer of the hypothalamus-hypophysis-testis axis, and show the reproductive endocrine disturbing effects. The reproductive endocrine disturbing effects of male maybe more severe than those of female.

Ma (2008)[100] **OBJECTIVE:** To study the endocrine disturbing effect of fluorin on Hypothalamus-Hypophysis-Testis axis in male rats. **METHODS:** A total of 36 Wister male rats weighting 60-70 g were randomly divided into group I (high fluoride group of F-100 mg/l), group II (low fluoride group of F- 30 mg/l), group III (control group with pure water), with 12 rats in each group. Fluoride was administered with drinking water for 8 weeks. Then the level of procreation hormone in serum was detected by RIA method. And the spermatozoa quality was analysized. **RESULTS:** There was difference between group I, group II and group III each other ($P < 0.05$) in body weight. As to right testis weight, there was difference between group I, group II and group III each other ($P < 0.05$). Epididymide organic coefficient in group II and group I were lower than that in group III ($P > 0.05$). Compared with group III, the counts amount of sperm and the rates of sperm mobility in group II and group I singnificantly increased ($P < 0.05$), and the rates of sperm aberration in group II and group I significantly decreased ($P < 0.05$), compared with group II, the sperm quality of group I descreased significantly ($P < 0.05$). The level of GnRH in three groups were significant difference between each groups ($P <0.05$). The level of FSH in three groups were significant difference between each groups ($P < 0.05$). The level of ICSH in three groups were no significant difference between each groups ($P > 0.05$). The level of T in Group I is significant lower than that of in Group II and Group III ($P < 0.05$). The level of E2 in Group I is significant higher than that of in Group II and Group III ($P < 0.05$).

Gupta (2007)[101] "The present study was undertaken to evaluate the effect of fluoride toxicity on the reproductive system of male rats. Sexually mature male Wistar rats were exposed to 2, 4, and 6 ppm sodium fluoride in their drinking water for 6 months

[100] Ma X[1], Cheng X, Li F, Guo J.[Experimental research on endocrine disturbing effect of fluorin on hypothalamus-hypophysis-testis axis in male rats]. Wei Sheng Yan Jiu. 2008 Nov;37(6):733-5. [Article in Chinese]

[101] Gupta RS[1], Khan TI, Agrawal D, Kachhawa JB. The toxic effects of sodium fluoride on the reproductive system of male rats. Toxicol Ind Health. 2007 Oct;23(9):507-13.

ad libitum. Sperm motility and density in cauda epididymis were assessed. Biochemical and histological analysis were performed in reproductive organs. Fluoride treatment brought about a significant decrease in the weight of testis, epididymis, and ventral prostate. The sperm motility and density were significantly reduced. There was a marked reduction in the number of primary spermatocyte, secondary spermatocyte, and spermatids. The Sertoli cell counts and their cross sectional surface areas were significantly decreased. The Leydig cell nuclear area and the number of mature Leydig cells were also significantly decreased. The protein content of the testis and epididymis were significantly reduced. Fructose in the seminal vesicles and cholesterol in testes were increased significantly. In conclusion, sodium fluoride administrated in drinking water of 2, 4, and 6 ppm concentration for 6 months to male rats adversely affected their fertility and reproductive system."

Jiang (2007)[102] OBJECTIVE: To study the damages of fluoride on the male reproductive system in rat testes. METHODS: A total of 30 male SD rats were randomly divided into control group, high, low dose fluorine treated groups, which were given normal saline ,20 mg/kg sodium fluoride, and 10mg/kg sodium fluoride respectively. After 39 days the change of the weight of rats and the number of sperms were observed. The change of telomerase reverse transcriptase(TERT) and proliferating cell nuclear antigen (PCNA) were observed by using in situ hybridization and radioimmunoassay respectively.RESULTS: The weight was (273.39 +/- 20.68), (240.00 +/- 21.39) g in NaF treated groups, which was lower than that in control group($P < 0.05$); The rate of TERT expression in germ cells of testes in NaF treated groups was (13.89 +/- 4.86)% and (6.33 +/- 4.42)% respectively, which was significantly lower than that in control group ($P < 0.05$). The rate of PCNA expression in germ cells of tests in NaF treated groups was (0.71 +/- 0.05)%, (0.60 +/- 0.08)% respectively, which also was significant lower than that in control group($P < 0.05$). The number of sperms was (18.31 +/- 1.20)10(10)/L, (9.17 +/- 1.38)10(10)/L, which was lower than that in control group ($P < 0.05$). CONCLUSION: Fluorine possibly damages the male reproductive system by reducing the expression of TERT and PCNA.

Oncu (2007)[103] (Note: Oncu's rats were given 0.7 mg/l NaF, the same as USPHS recommended "This experiment was designed to investigate the histological and lipid peroxidation effects of chronic fluorosis on testes tissues of first- and second-generation rats. Sixteen virgin female Wistar rats were mated with eight males (2:1) for approximately 12 h to obtain first-generation rats. Pregnant rats were divided into two groups: controls and fluoride-given group, each of which containing five rats. Pregnant rats in the fluoride-given group were exposed to a total dose of 30 mg/l sodium fluoride (NaF) in commercial drinking water containing 0.07 mg/l of NaF throughout the gestation and lactation periods. After the lactation period, the young

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[102] Jiang Q[1], Song XK, Cui QH, Chen LJ. [Effect of fluoride on expression of telomerase reverse transcriptase expression and proliferating cell nuclear antigen in germ cells of rats' testes]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2007 Feb;25(2):96-9. [Article in Chinese]

[103] Oncü M[1], Kocak A, Karaoz E, Darici H, Savik E, Gultekin F. Effect of long-term fluoride exposure on lipid peroxidation and histology of testes in first- and second-generation rats. Biol Trace Elem Res. 2007 Sep;118(3):260-8.

animals (first generation, F1) were exposed to the same dose of NaF in drinking water for 4 months. At the end of the 4 months of experimental period, nine randomly chosen male rats (F1) were killed and testes tissues were taken for histopathological and biochemical analysis. The remaining eight female rats were mated with four males (2:1) for approximately 12 h to obtain second-generation rats. Six female were identified as pregnant and treated with similarly throughout the gestation and the lactation periods. After the lactation period, the young male animals (second generation, F2) were also treated in the same way for 4 months. At the end of the 4 months of experimental period, nine randomly chosen male rats (F2) were killed and testes tissues were collected for histopathological and biochemical analysis. The rats in the control group were applied the same procedure without NaF administration. In biochemical analysis of the fluoride given F1 and F2 rats, it has been found that plasma fluoride levels and testes thiobarbituric acid reactive substance levels were significantly increased when compared with the control group. In F1 and F2 rats, similar histopathological changes were observed. In both groups, spermatogenesis was severely reduced. Spermatogonia and primary spermatocytes were normal, however, there was a widespread degeneration in other spermatogenic cell lines of the seminiferous epithelium. The histological structures of the Sertoli and interstitial Leydig cells were normally observed. It is concluded that chronic fluorosis exposure leads to a remarkable destruction in testes tissues of F1 and F2 rats via lipid peroxidation."

Dvoráková-Hortová K (2007)[104] Increasing infertility, due to pathological changes on sperm, has become a serious issue. Eco-toxicological effect of rising concentration of fluorides can be enhanced in the presence of aluminium ions by forming fluorometallic complexes, analogues of phosphate groups that interfere with the activity of G-proteins and P-type ATPases, which are part of several signalling pathways during sperm maturation. In order for sperm to gain fertilizing ability, they must undergo in the female reproductive tract, capacitation that includes tyrosine phosphorylation and consequent actin polymerization. The present paper reports the findings of 3-month oral toxicity in mice of fluorides at the concentrations 0, 1, 10, and 100ppm and their synergic action with aluminium at dose of 10ppm. There were no mortalities, clinical signs of discomfort or body weight loss during the experiment. The analysis revealed, for the concentrations of 10 and 100ppm, abnormalities of spermatogenesis and ability of epididymal spermatozoa to capacitate in vitro, as the result of decreased sperm head tyrosine phosphorylation and actin polymerization. The enhancing overload caused by fluorides represents a potential factor, having an impact on function of sperm, hence contributing to a growing infertility in the human population.

Zakrzewska (2006)[105] "RESULTS: The semen was diluted in 0.9% NaCl and was found

[104] Dvoráková-Hortová K[1], Sandera M, Jursová M, Vasinová J, Peknicová J. The influence of fluorides on mouse sperm capacitation. Anim Reprod Sci. 2008 Oct;108(1-2):157-70. Epub 2007 Aug 6.

[105] Zakrzewska H, Udala J. (2006). [In vitro influence of sodium fluoride on adenosine triphosphate (ATP) content in ram semen]. [Article in Polish]. Ann Acad Med Stetin. 52 Suppl 1:109-11

to contain 12.4 micromol ATP 10-(-9) spermatozoa. ATP content was reduced with rising concentrations of NaF: by 74.6% at 20 tmol/L; by 75.5% at 100 micromol/L; by 90.8% at 200 imol/L; and by 99.9% at 10(5) micromol/L. The correlation between ATP content and sperm motility was significant (r = 0.4990). There was no correlation between ATP content and sperm density."

Krasowska (2004)[106] "Previous work has shown that a high fluoride intake in rodents leads to histopathological changes in the germinal epithelium of testes that is associated with zinc deficiency. The purpose of this study was to determine whether supplemental dietary Zn would protect against testicular toxicity induced by fluoride in a small rodent, the bank vole. The 4-month exposure period to fluoride (200 microg/ml of drinking water) induced histopathological changes (hemorrhage in interstitium, necrosis and apoptosis in seminiferous tubule epithelium) which were accompanied by decreased testicular zinc concentration and increased lipid peroxidation. Supplemental dietary zinc (110-120 microg/g) together with fluoride treatment resulted in complete reversal of the fluoride-mediated effects. However, supplemented dietary Zn did not affect the accumulation of fluoride in the testes and bone. These data suggest that a zinc-enriched diet protects seminiferous tubules against fluoride toxicity by preventing the fluoride-induced testicular zinc deprivation."

Zakrzewska (2002)[107] "The activities of androgen-dependent enzymes—acid phosphatase (ACP), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (y-GT-10S)—decreased significantly when the ejaculate was treated with NaF at concentrations of 20, 100, 200 µmol/L (0.38; 1.9; 3.8 ppm F-), but they returned to the initial value of the control at 0.1 mol/L (1900 ppm F-). . . . These changes undoubtedly affect the physiological functions of the sperm."

Ghosh (2002)[108] "This study examined the effect of sodium fluoride, a water pollutant important through the world, including India, on testicular steroidogenic and gametogenic activities in relation to testicular oxidative stress in rats. Sodium fluoride treatment at 20mg/kg/day for 29 days by oral gavage resulted in significant diminution in the relative wet weight of the testis, prostate, and seminal vesicle without alteration in the body weight gain. Testicular delta(5),3beta-hydroxysteroid dehydrogenase (HSD) and 17beta-HSD activities were decreased significantly along with significant diminution in plasma levels of testosterone in the fluoride-exposed group compared to the control. Epididymal sperm count was decreased significantly in the fluoride-treated group and qualitative examination of testicular sections

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[106] Krasowska A[1], Włostowski T, Bonda E. Zinc protection from fluoride-induced testicular injury in the bank vole (Clethrionomys glareolus). Toxicol Lett. 2004 Mar 7;147(3):229-35.

[107] Zakrzewska H, et al. (2002). In vitro influence of sodium fluoride on ram semen quality and enzyme activities. Fluoride 35: 153-160.

[108] Ghosh D[1], Das Sarkar S, Maiti R, Jana D, Das UB. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod Toxicol. 2002 Jul-Aug;16(4):385-90.

revealed fewer mature luminal spermatozoa in comparison to the control. The seminiferous tubules were dilated in treated animals. Fluoride treatment was associated with oxidative stress as indicated by an increased level of conjugated dienes in the testis, epididymis, and epididymal sperm pellet with respect to control. Peroxidase and catalase activities in the sperm pellet were decreased significantly in comparison to the control. The results of this experiment indicate that fluoride at a dose encountered in drinking water in contaminated areas exerts an adverse effect on the male reproductive system and this effect is associated with indicators of oxidative stress."

Susheela (1996)[109] "OBJECTIVE: The present study focuses on serum testosterone concentrations in patients with skeletal fluorosis, in order to assess the hormonal status in fluoride toxicity. METHODS: Serum testosterones were compared for patients afflicted with skeletal fluorosis (n = 30) and healthy males consuming water containing less than 1 ppm fluoride (Control 1, n = 26) and a second category of controls (Control 2, n = 16): individuals living in the same house as the patients and consuming same water as patients but not exhibiting clinical manifestations of skeletal fluorosis. RESULTS: Circulating serum testosterones in skeletal fluorosis patients were significantly lower than those of Control 1 at $p < 0.01$. Testosterone concentrations of Control 2 were also lower than those of Control 1 at $p < 0.05$ but were higher than those of the patient group. CONCLUSIONS: Decreased testosterone concentrations in skeletal fluorosis patients and in males drinking the same water as the patients but with no clinical manifestations of the disease compared with those of normal, healthy males living in areas nonendemic for fluorosis suggest that fluoride toxicity may cause adverse effects in the reproductive system of males living in fluorosis endemic areas."

Chinook (1994)[110] "Fluoride-treated sperm [4,750 ppm for 20 minutes] exhibited a high percent of morphologic abnormalities, including a large number (10.59%) of elongated heads and 2.1% amorphous heads. The tail also revealed splitting (2.19%), coiling (11.6%) and deflagellation (22.43%). A few sperm had bent necks, and 16.75% of spermatozoa showed a diminutive acrosome. . . . These changes may have caused loss of membrane integrity and reduced metabolic activity, which ultimately resulted in deterioration of forward progression rating. The treatment caused a significant enhancement in poor to fair forward progression and failure of good and excellent forward progression, leading to a significant decline in sperm motility. . . . The depleted sperm GSH in the present investigation strongly suggests that, like several exogenous compounds, fluoride is largely dependent upon glutathione for detoxification."

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[109] Susheela AK1, Jethanandani P., Circulating testosterone levels in skeletal fluorosis patients. J Toxicol Clin Toxicol. 1996;34(2):183-9.

[110] Chinoy NJ, Narayana MV. (1994). In vitro fluoride toxicity in human spermatozoa. Reprod Toxicol. 8(2):155-9.

Chubb (1985a)[111] “Our studies indicate that 3 ppm fluoride ions significantly inhibit testosterone secretion by rat testes perfused in vitro. . . . In conclusion, Oxypherol-E.T. contains contaminants that are toxic to endocrine organs. Fluoride ion may be the primary endocrine toxicant.”

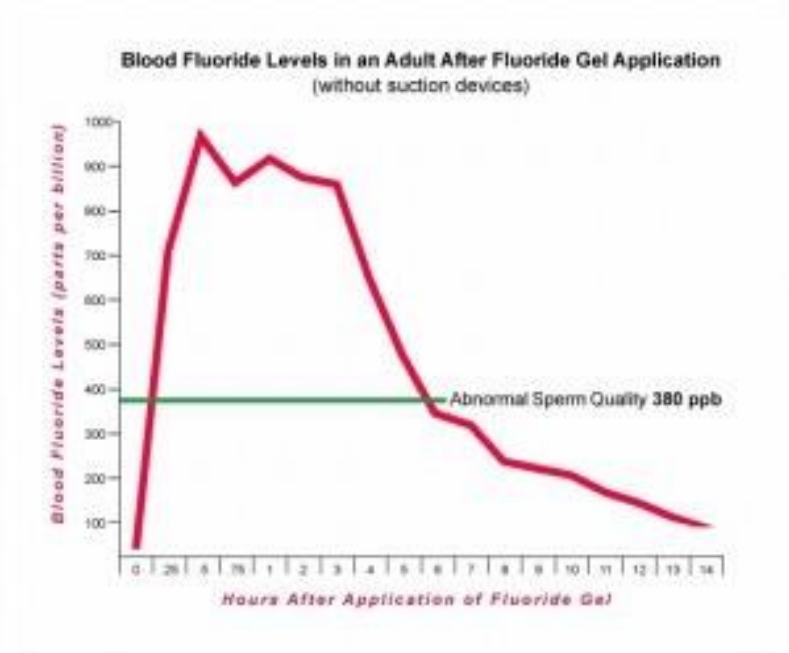
REVIEW BY FAN (2011)[112] "The enhancing overload caused by fluorides represents a potential factor, having an impact on function of sperm, hence contributing to a growing infertility in the human population.” (Animal Reproduction Science, 2008

“Male infertility is responsible for about 50% of the fertility problems that couples face. Infertility in males is often the result of reduced sperm court, abnormal sperm quality (e.g., reduced motility and altered morphology), or altered levels of sex hormones (e.g., reduced testosterone). A review of over 100 studies of sperm density from 1938 to 1996 found that human sperm count has significantly declined in North America and Europe since the 1940s. (Swan 2000) While the causes of this decline are not entirely known, fluoride exposure — particularly from high-concentration topical fluoride gels — must be considered as one of the potential contributing factors.

“In 2002 and again in 2006, researchers from Poland reported that exposing ram semen to 0.38 parts per million (20 umol/L) of fluoride for 5 hours was sufficient to “cause a statistically significant decrease in the motility of spermatoza and the number of intact acrosomes.” (Zakrzewska 2002). As the authors noted, these changes would “undoubtedly affect the physiological function of the sperm.” Prior to the Polish team’s findings, researchers from Texas found that infusing testis with higher, but still relatively modest, levels of fluoride (4.75 ppm) “unequivocally” inhibited the synthesis of testosterone. (Chubb 1985).

[111] Chubb C. (1985a). Reversal of the endocrine toxicity of commercially produced perfluorochemical emulsion. Biology of Reproduction 33(4):854-8.

[112] http://fluoridealert.org/issues/health/fertility/



"The Polish team's findings are of particular importance when considering that from the 1960s to the 1990s, the use of high-concentration topical fluoride gels produced blood concentrations in boys and men that far exceeded 0.38 ppm. In tests on both children and adults, the use of topical fluoride gels at the dental office has been found to produce blood fluoride concentrations as high as 1.2 ppm, or four times higher than the concentration found to damage sperm. (Ekstrand 1980, 1981). Further, the blood fluoride concentrations have been found to exceede 0.38 ppm for up to six hours after treatment (longer than the length of time that the Polish researchers exposed the semen). Although most dentists now use precautionary procedures (e.g., suction devices) to reduce blood fluoride concentrations following application of fluoride gels, available data shows that children will still routinely ingest enough fluoride from topical gels to reach blood fluoride concentrations exceeding 0.38 ppm.

"Consistent with the in vitro research, over 60 animal studies have found that fluoride adversely impacts the male reproductive system. The effects — which have been observed in rats, mice, chickens, and rabbits — include: (1) decreases in testosterone levels; (2) reduced sperm motility; (3) altered sperm morphology; (4) reduced sperm quantity; (5) increased oxidative stress; (6) and reduced capacity to breed. While the studies have generally used high doses, many of the studies have found effects at dosages that would produce blood fluoride concentrations far lower than the concentrations used in the in vitro research. See, e.g., Sun (2010); Dvoráková-Hortová (2008); Sharma (2008); Reddy (2007); Gupta (2007); Pushpalatha (2005). In one of the few studies to report blood fluoride concentrations, Mexican researchers reported that blood fluoride levels of 0.2 to 0.26 ppm for an eight week period caused increased oxidative stress, reductions in sperm motility and reduced fertility in male rats. Izquierdo-Vega, et al. (2008).

"While some studies have not found any effects of high fluoride dosages on the reproductive functions of male rats , these studies represent the distinct minority in the field. (Sprando & Collins 1996, 1997, and 1998). One possible explanation for the discrepancy in findings is the nutritional health of the tested animals. As with many other areas of fluoride research, nutritional deficiencies (e.g., protein) unequivocally exacerbate fluoride's reproductive effects, whereas nutritional supplementation (e.g., protein or anti-oxidants such as vitamin C) can significantly prevent or ameliorate these effects.

"Consistent with the in vitro and animal research, studies of human populations have reported associations between fluoride exposure and damage to the male reproductive system. Most notably, a scientist at the Food & Drug Administration reported in 1994 that populations in the United States with more than 3 ppm fluoride in their water had lower "total fertility rates" than populations with lower fluoride levels. (Freni 1994). While 3 ppm is a higher concentration than used in water fluoridation programs (0.7 to 1.2 ppm), it is still considered a "safe" level by the EPA. To date, no U.S. health agency has attempted to replicate Freni's findings. However, three studies of highly fluoride-exposed populations in China and India have found that high fluoride exposure is associated with reduced male fertility. (Chen 1997; Liu 1988; Neelam 1987). In addition, five studies from China, India, Mexico, and Russia.have found that high-fluoride exposure is associated with reduced male testosterone levels. (Hao 2010; Ortiz 2003; Susheela 1996; Michael 1996; Tokar 1977)." End of FAN quote.

G. ENTEROENDOCRINE (See the Pancreas for Pancreatic enteroendocrine)

Wikipedia: Enteroendocrine cells are specialized endocrine cells of the gastrointestinal tract and pancreas. They produce gastrointestinal hormones or peptides in response to various stimuli and release them into the bloodstream for systemic effect, diffuse them as local messengers, or transmit them to the enteric nervous system to activate nervous responses.[1][2] Enteroendocrine cells of the intestine are the most numerous endocrine cells of the body.[3][4][5] In a sense they are known to act as chemoreceptors, initiating digestive actions and detecting harmful substances and initiating protective responses.[6][7] Enteroendocrine cells are located in the stomach, in the intestine and in the pancreas. Intestinal

enteroendocrine cells are not clustered together but spread as single cells throughout the intestinal tract.[8]Hormones secreted include somatostatin, motilin, cholecystokinin, neurotensin, vasoactive intestinal peptide, and enteroglucagon.[9]

Searches did not readily find studies specifically evaluating the enteroendocrine cells and fluoride. We do have studies on fluoride's effect on the gastrointestinal cells as a group. If gastrointestinal cells are being harmed with fluoride, it is reasonable to expect enteroendocrine cells to be similarly involved.

Social (2010)[113] "Results reveal that (1) the urine fluoride levels decreased in 67% and 53% of the pregnant women respectively, who attended ANCs (antenatal clinic to reduce fluoride intake and improve diet) during 1st and 2nd trimester of pregnancy. (2) An increase in Hb upon withdrawal of fluoride followed by nutritional intervention in 73% and 83% respectively has also been recorded. (3) Body mass index (BMI) also enhanced. (4) The percentage of pre-term deliveries was decreased in sample group compared to control. (5) Birth weight of babies enhanced in 80% and 77% in sample group women who attended ANC in 1st and 2nd trimester respectively as opposed to 49% and 47% respectively in the control group. (6) The number of low birth weight babies was reduced to 20% and 23% respectively in sample as opposed to 51% and 53% in control groups."

NRC (2006)[114] "It is important to realize that GI effects depend more on the net concentration of the aqueous solution of fluoride in the stomach than on the total fluoride dose in the fluid or solid ingested. The presence of gastric fluids already in the stomach when the fluoride is ingested can affect the concentration of the fluoride to which the gut epithelium is exposed. The residual volume of stomach fluid ranges between 15 and 30 mL in people fasting overnight (Narchi et al. 1993; Naguib et al. 2001; Chang et al. 2004). Such volumes would decrease the fluoride concentration of a glass of drinking water by only about 10%. In Table 9-1, the concentrations of fluoride in the stomach were estimated from the mean reported fluoride exposures. A dilution factor was used when it was clear that the subjects already had fluid in their stomach. The results from the water fluoridation overfeed reports (concentrations of fluoride in the stomach between 20 and 250 mg/L) indicate that GI symptoms, such as nausea and vomiting, are common side effects from exposure to high concentrations of fluoride.

"Fluoride supplements are still routinely used today in areas where natural fluoride in the drinking water falls below 0.7 mg/L. In an early clinical trial using fluoride supplements, Feltman and Kosel (1961) administered fluoride tablets containing 1.2

[113] Susheela AK et al, Effective interventional approach to control anaemia in pregnant women. Current Science, May 25, 2010. 98(10):1320-30.
[114] National Research Council. (2006). Fluoride in Drinking Water: A Scientific Review of EPA's Standards. National Academies Press, Washington D.C. p 229-230.

mg of fluoride or placebo tablets to pregnant mothers and children up to 9 years of age. They determined that about 1% of the subjects complained of GI symptoms from the fluoride ingredient in the test tablets. If it is assumed that the stomach fluid volume after taking the fluoride supplement was approximately 250 mL, the concentration to which the stomach mucosal lining was exposed was in the neighborhood of 5 mg/L. GI effects appear to have been rarely evaluated in the fluoride supplement studies that followed the early ones in the 1950s and 1960s. Table 9-1 suggests that, as the fluoride concentration increases in drinking water, the percentage of the population with GI symptoms also increases. The table suggests that fluoride at 4 mg/L in the drinking water results in approximately 1% of the population experiencing GI symptoms."

Connett (2012) provided an overview of fluoride and gastric mucosa: "When fluoride has been used (at doses of 18-34 mg/day) as an experimental treatment for osteoporosis, gastric pain is one of the two main side effects consistently encountered. To better understand how fluoride causes this effect, researchers have sought to determine how fluoride affects the tissue that lines the gastrointestinal tract.

In a study published in the British Medical Journal, the researchers gave a *single* dose of 20 mg/F to 12 healthy volunteers and then examined, both microscopically and macroscopically, the impact on the gastric mucosa. The examination revealed that the fluoride dose caused erosions (petechiae) in the stomach of *all* the subjects tested, with six of the subjects having similar effects in the antrum as well. Other findings were as follows:
"In four subjects a layer of clotted blood was found over a large part of the gastric mucosa… Three components of the gastric mucosa were affected by fluoride: the surface epithelium, the gastric pits, and the superficial stroma. The damaged epithelial cells were smaller than undamaged ones, and the vacuoles containing mucus were reduced in size or had disappeared. The most severely damaged epithelium was disrupted or totally lost. The most characteristic changes in the gastric pits were irregular dilation and flattening of the epithelial cells. There was also a noticeable loss of mucin."
SOURCE: Spak CJ, et al. (1989). Tissue response of gastric mucosa after ingestion of fluoride. British Medical Journal 298:1686-87. [See study]

Despite the fact that tissue damage was found in all 12 volunteers, only 4 of the volunteers experienced nausea. Thus, "using nausea as the first sign of fluoride toxicity might not be valid as all subjects showed mucosal damage."

In a follow-up study, published in 1990, the authors examined the impact of lower doses of fluoride to determine whether the use of self-applied topical gels could cause damage to children's gastric system. In the study, the volunteers ingested a single dose of just **3 to 9 mg** of fluoride, which is considerably lower than what some people ingest from higher-concentration professional fluoride gels. Despite using low

doses, the authors again found significant damage to the gastric mucosa. They described this damage as follows:
"After F exposure, histopathological changes were found in nine out of ten patients. The surface epithelium of the gastric mucosa showed the greatest effects: In two cases, there was a slight dilation of the gastric pits and a focal loss of surface epithelium. In some cases, the mucus-containing intercellular vacuoles were reduced in size, and focal hemorrhages within the epithelium occurred."
SOURCE: Spak CJ, et al. (1990). Studies of human gastric mucosa after application of 0.42% fluoride gel. Journal of Dental Research 69:426-9.

Interestingly, the authors note that they "could not find any correlation between the presence of mucosal injuries and the size of the ingested F dose." Based on this, they suggest that individual variability to fluoride may be a more important predictor of fluoride-induced gastric damage when low levels of fluoride are ingested. As they note: "The various reactions of the mucosa to F exposure are most likely due to individual variations in gastric fluid volume, gastric pH, and motility and mucosal resistance."
Such findings emphasize the difficulty of determining a uniform "safe" fluoride dose for an entire population. Indeed, if significant variability to fluoride is observed among 10 otherwise healthy humans, the variability is likely to be quite vast when studying the population as a whole, especially when including those with diseases that render one particularly susceptible to fluoride toxicity.

EXCERPTS FROM STUDIES EXAMINING FLUORIDE'S EFFECT ON GASTRIC MUCOSA IN HUMANS

"In a prospective case controlled study, we evaluated the adverse effects of long-term fluoride ingestion on the gastrointestinal tract. Ten patients with otosclerosis who were receiving sodium fluoride 30 mg/day for a period of 3-12 months, and 10 age- and sex-matched healthy volunteers were included… Seven subjects (70%) ingesting fluoride had abdominal pain, vomiting, and nausea. Petechiae, erosions, and erythema were seen on endoscopy in all the subjects, but not in the controls. Histological examination of the gastric antral biopsy showed chronic atrophic gastritis in all the subjects but in only one (10%) healthy volunteer. Scanning electron microscopic examination showed "cracked-clay" appearance, scanty microvilli, surface abrasions, and desquamated epithelium in the subjects ingesting fluoride, but not in the controls. We conclude that long-term fluoride ingestion is associated with a high incidence of dyspeptic symptoms as well as histological and electron microscopic abnormalities."
SOURCE: Das TK, et al. (1994). Toxic effects of chronic fluoride ingestion on the upper gastrointestinal tract. Journal of Clinical Gastroenterology 18(3):194-9.
"In a randomized double-blind study with two parallel groups of 10 male healthy volunteers each the response of gastric mucosa after a 7 days ingestion of sodium fluoride tablets (NaF) or sodium monofluorophosphate tablets (MFP) was compared. Gastroscopic evaluations were performed before treatment, day 1 and day 7… In the MFP-group no severe gastric lesions were observed, whereas in the NaF-group in 7 of

the 10 subjects significant gastric mucosal lesions including acute hemorrhages and free blood in the gastric lumen were found. The differences of the lesions scores in both groups were statistically significant (p = 0.0015)… In summary, under the experimental conditions used MFP is well tolerated by the stomach while NaF produces significant gastric mucosal lesions."
SOURCE: Muller P, et al. (1992). Sodium fluoride-induced gastric mucosal lesions: comparison with sodium monofluorophosphate. Z Gastroenterol. 30(4):252-4.
"Dental prophylaxis with APF gels (1.23%) may cause gastric distress as a side-effect. This gastric irritation is probably due to a direct toxic effect of fluoride (F), swallowed in conjunction with the treatment, on the gastric mucosa. The aim of the present study was to investigate whether–and to what extent–a dental treatment with 3 g of a 0.42%-F gel could affect the gastric mucosa due to inadvertent swallowing of the gel. Ten subjects underwent a control gastroscopy, and two weeks later, a second gastroscopy was performed two h after a F gel treatment. During the gastroscopy, the mucosa was examined and the injuries graded according to an arbitrary scale. Four biopsies of the antral and corpus regions of the stomach were taken and evaluated histologically. The mean (+/- SD) amount of F retained after the application was 5.1 +/- 2.1 mg, i.e., 40% of the applied amount of F. Petechiae and erosions were found in the mucosa in seven of the ten patients. The histopathological evaluation revealed changes in nine of ten patients, with the surface epithelium as the most affected component of the mucosa. The present study clearly shows that a treatment with a F gel of rather low F concentration may result in injuries to the gastric mucosa. The importance of current recommended guidelines so that the amount of F swallowed during a gel application can be minimized is emphasized. From a toxicological standpoint, the use of a low-F gel instead of a 1.23%-F gel in small children is recommended for avoidance of adverse gastric effects."
SOURCE: Spak CJ, et al. (1990). Studies of human gastric mucosa after application of 0.42% fluoride gel. Journal of Dental Research 69:426-9.
"We studied the response of the gastric mucosa after a single dose of fluoride. Twelve healthy volunteers (age range 22-45, four men and eight women) underwent two endoscopies after overnight fasts. One endoscopy was a control and the other was performed two hours after subjects ingested 20 ml sodium fluoride solution containing 20 mg fluoride (53 mmol/l)… After taking fluoride all subjects had petechiae or erosions (graded 3 or 4) in the body of the stomach and six had changes (graded 1-4) in the antrum. No petechiae or erosions were recorded in the oesophagus or the duodenum. In four subjects a layer of clotted blood was found over a large part of the gastric mucosa… Three components of the gastric mucosa were affected by fluoride: the surface epithelium, the gastric pits, and the superficial stroma. The damaged epithelial cells were smaller than undamaged ones, and the vacuoles containing mucus were reduced in size or had disappeared. The most severely damaged epithelium was disrupted or totally lost. The most characteristic changes in the gastric pits were irregular dilation and flattening of the epithelial cells. There was also a noticeable loss of mucin. Our study showed that one ingestion of fluoride at a dose used to treat osteoporosis affects the gastric mucosa… Symptoms like nausea and vomiting are not unusual when fluoride is used to treat osteoporosis. They also occur occasionally when high doses are used for dental prophylaxis. In our study only four subjects developed

nausea, which suggests that using nausea as the first sign of fluoride toxicity might not be valid as all our subjects showed mucosal damage."
SOURCE: Spak CJ, et al. (1989). Tissue response of gastric mucosa after ingestion of fluoride. British Medical Journal 298:1686-87.

H. Paraganglia

"Paranganglia," refers to the groups of chromaffin cells associated with the sympathetic system. "Paraganglia are neuroendocrine organs mainly comprising cells that take their origin in the neural crest. They secrete catecholamines or indolamines and peptides. They are divided into two groups, associated with the sympathetic or parasympathetic nervous systems."[115]

Research specifically evaluating fluoride's effect on paraganglial tissues is not readily available at this time from our search.

I. Pituitary Gland

The pituitary gland is about the size of a pea (0.018 oz) and sits at the base of the brain. The anterior pituitary regulates several physiological processes including stress, growth, reproduction, blood pressure, metabolism, salt/water regulation of kidneys, temperature, pain relief and lactation, while the intermediate lobe synthesizes and secretes melanocyte-stimulating hormone and the posterior lobe is functionally connected to the hypothalamus.

The effects of fluoride pesticides on the pituitary gland are reported at http://www.fluoridealert.org/wp-content/pesticides/effects.endocrine.pituitary.htm

J. Placenta.

The phrase "buyer beware" comes to mind (in a sad guilty way) when searching studies for the effect of fluoride on the placenta, very few exist. In our capitalistic society we expect the buyer, the patient, to be responsible for purchase, use, and safety, especially of fluoride. Apparently we adults expect the fetus to do adequate and quality research on the effects of fluoride on the placenta and themselves, because we adults sure have not. Why have we adults failed to protect the unborn?

In a 1952 issue of *Science* magazine,[116] Harold C. Hodge (chief toxicologist for the US Army's Manhattan Project) reported that women who drank artificially fluoridated water

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[115] Endocrine Pathology:: Differential diagnosis and Molecular Advances. Lloyd RV Editor. Chapter 12, Adrenal Medulla and Paraganglia by McNicol AM.

[116] Gardner DE, Smith FA, Hodge HC, Overton DE, Feltman R. The fluoride concentration of placental tissue as related to fluoride content in drinking water. *Science*. 1952;115(2982):208–209.
See also: Chlubek D, Poreba R, Machalinski B. Fluoride and calcium distribution in human placenta. *Fluoride*. 1998 31(3):131–136.

(1.0–1.2 ppm fluoride) averaged 2.09 ppm fluoride in their placentas, compared with 0.74 ppm fluoride in the placentas of women who drank nonfluoridated water (0.06 ppm fluoride). Maternal blood fluoride levels were also nearly three times higher (0.040 vs. 0.014 ppm).

Tskitishvili (2010)[117] "Oxidative stress with elevated intracellular Ca^{2+} concentration as well as endothelial dysfunction is a component of pre-eclampsia. Our aim was to investigate the oxidative stress-dependent expression of Endoglin and Ca^{2+}-binding S100B protein from villous and amniotic tissue cultures, and to assess sEng expression from S100B protein-stimulated endothelial cells. We initially examined Endoglin and Hydroxy-nonenal-(HNE)-modified proteins in the placentas and amnion obtained from women with pre-eclampsia (n = 8), and healthy controls (n = 8) by immunohistochemistry. To examine oxidative stress and the S100B protein effect on sEng expression from endothelial cells, normal villous and amniotic tissue cultures were stimulated by 4-HNE, sodium fluoride and xanthine/xanthine oxidase, whereas human umbilical vein endothelial cell cultures were treated with S100B protein in a dose- and time-dependent manner at 37°C in an environment of 95% air and 5% of CO_2. Culture supernatants were assessed using ELISA. Cell viability was determined using MTS assay. The concentrations of sEng and S100B protein were significantly increased in the villous and amniotic tissue culture supernatants under oxidative stress. S100B protein-stimulated endothelial cells released sEng into conditioned media with a significantly higher expression levels at a concentration of 200 pM–20 nM S100B by 2 h, whereas treated with 200 nM of S100B endothelial cells significantly expressed sEng by 12 h and stimulated the cell proliferation by the same period of time. Our findings show that oxidative stress affects sEng and S100B protein expression from villous and amniotic tissues, and picomolar and low nanomolar concentrations of S100B protein significantly up-regulate sEng release from endothelial cells leading to endothelial dysfunction."

Dlugosz (2009) "The aim of the study was to investigate the role of oestrogens in free radical detoxication upon exposure to fluoride. Interactions between xenobiotics and oestrogens need to be investigated, especially as many chemicals interact with the oestrogen receptor. It is still unknown whether free radical-generating xenobiotics can influence the antioxidative ability of oestradiol (E(2)). In an in vitro examination of human placental mitochondria, thiobarbituric active reagent species (TBARS), hydroxyl radical ((*)OH) generation and protein thiol (-SH) groups were detected. 17beta-E(2) was examined in physiological (0.15-0.73 nM) and experimental (1-10 microM) concentrations and sodium fluoride (NaF) in concentrations of 6-24 microM. E(2) in all the concentrations significantly decreased lipid peroxidation measured as the TBARS level, in contrast to NaF, which increased lipid peroxidation. Lipid

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Sastry GM, Mohanty S, Rao P. Role of placenta to combat fluorosis (in fetus) in endemic fluorosis area. *Natl J Integr Res Med*. 2010 Oct–Dec;1(4):16–19.

[117] E. Tskitishvili[1,3], N. Sharentuya[1], K. Temma-Asano[1], K. Mimura[1], Y. Kinugasa-Taniguchi[1], T. Kanagawa[1], H. Fukuda[1], T. Kimura[1], T. Tomimatsu[1] and K. Shimoya· Oxidative stress-induced S100B protein from placenta and amnion affects soluble Endoglin release from endothelial cells. Mol Hum Reprod. 2010 Mar;16(3):188-99. doi: 10.1093/molehr/gap104. Epub 2009 Nov 25.

peroxidation induced by NaF was decreased by E(2). The influence of E(2) on (*)OH generation was not very significant and depended on the E(2)concentration. The main mechanism of E(2) protection in NaF exposure appeared to be connected with the influence of E(2)on thiol group levels, not (*)OH scavenging ability. The E(2) in concentrations 0.44-0.73 nM and 1-10 microM significantly increased the levels of -SH groups, in contrast to NaF, which significantly decreased them. E(2) at every concentration reversed the harmful effects of NaF on -SH group levels. No unfavourable interactions in the influence of E(2) and NaF on TBARS production, (*)OH generation, or -SH group levels were observed. The results suggest that postmenopausal women could be more sensitive to NaF-initiated oxidative stress."

Srednicka (2007)[118] "The interactions in free radicals processes between cyclosporine A (CsA) and sodium fluoride (NaF) on in vitro model human placental mitochondria were evaluated. The level of malondialdehyde, hydroxyl radical generation and concentration of sulfhydryl groups of protein was measured. The results showed that CsA with NaF did not give any toxicological interactions with NaF in the area of measured parameters.

Hassunuma (2007)[119] Little information is available on the pathogenesis of fluorosis during the fetal and initial postnatal period. In the present study, female rats received 0 (control), 7 or 100 ppm of sodium fluoride in drinking water, one week before breeding and throughout gestation and nursing periods. The hemimandibles of the offspring were collected at 0, 7 and 14 days of postnatal life (n = 5) and processed for morphological analyses by light and electron microscopy, immunohistochemical analysis for amelogenin and morphometric study of enamel matrix and ameloblasts of incisors. The results showed a decrease in matrix production at the secretory phase at all study periods for the 100 ppm group. In this same group, the secretory ameloblasts showed reduction of enamel matrix secretion, disorganization of mitochondrial crests, large vacuoles at the apical portion of the cytoplasm, retention of intracisternal material and dilatation of some cisterns in the rough endoplasmic reticulum. In the groups of animals aged 7 and 14 days, analysis of variance showed significant reduction ($p<0.05$) in cytoplasmic volume of 23.80% and 24.75%, respectively, in relation to the control group. The smooth-ended maturation ameloblasts exhibited a large number of vacuoles with electron-dense endocytic matrix, suggesting a delay in the resorption process. Immunohistochemical analysis showed no difference in the intensity and labeling pattern of the enamel matrix in any study group. Interestingly, in offspring at the age of 14 days for the 7 ppm group, there was an increase in the matrix length at the secretory phase. Therefore, part of the excessive dose of sodium fluoride given to the mother in drinking water can reach the offspring through the placenta and mother's milk, causing morphological

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[118] Srednicka D1, Długosz A., Interactions in free radicals processes between cyclosporine A and sodium fluoride. Acta Pol Pharm. 2007 Nov-Dec;64(6):503-8.

[119] Hassunuma RM1, Zen Filho EV, Ceolin DS, Cestari TM, Taga R, de Assis GF. Ultrastructural and immunohistochemical study of the influence of fluoride excess on the development of rat incisor tooth buds. J Appl Oral Sci. 2007 Aug;15(4):292-8.

changes in ameloblasts and suggesting a reduction in secretion and a delay in matrix resorption.

Toyama (2001)[120] "This study sought to obtain a precise profile of fluoride concentrations at and near the neonatal line in deciduous incisors and canines from the naturally fluoridated area (1.0--1.3 parts/10(6) F in drinking water) of West Hartlepool and the non-fluoridated area (less than 0.1 parts/10(6) F in drinking water) of Leeds in England. An abrasive microsampling method was used to determine the distribution of fluoride and phosphorus concentrations. The profile of fluoride concentrations in 100-microm layers before and after the neonatal line, that is, in the prenatal and postnatal enamel, were significantly higher in teeth from the fluoridated than non-fluoridated areas. It was concluded that the fact that the fluoride concentrations were about the same prenatally and postnatally in deciduous enamel obtained from the fluoridated and non-fluoridated areas indicates that fluoride enters the prenatal deciduous enamel and that it is transferred through the placenta."

Li (1999)[121] "Whole embryo rotated culture technique was used to investigate the toxicity of combination of selenium, fluoride and arsenic on rat embryos at day 9.5 of gestation. The result of factorial analysis (3 x 3 x 3) showed that the main effect of combination of selenium, fluoride and arsenic on the developmental toxicity was synergistic. The mixtures with different level of these three chemicals in combination could result in different developmental toxicity. The low level combinations mainly caused teratogenic effect, and the high level combinations(selenium 2.0 micrograms + fluoride 10 micrograms + arsenic 1.0 microgram/ml culture media) caused lethal effect. The results suggested that the disorders of yolk-sac placenta in structure and function were one of teratogenic mechanisms for the combination of selenium, fluoride and arsenic."

Flores-Herrera (1999)[122] "This report describes an ATP-diphosphohydrolase activity associated with the inner membrane of human term placental mitochondria. An enriched fraction containing 30 per cent of the total protein and 80 per cent of the total ATP-diphosphohydrolase activity was obtained from submitochondrial particles. ATP-diphosphohydrolase activity was characterized in this fraction. The enzyme had a pH optimum of 8 and catalysed the hydrolysis of triphospho- and diphosphonucleosides other than ATP or ADP. Pyrophosphate was also hydrolysed, but AMP or other monoester phosphates were not. The activity of ATP-diphosphohydrolase was dependent on Mg(2 +), Ca(2 +)or Mn(2 +)and the enzyme substrate was the cation-nucleotide complex. An excess of free cation produced inhibition.ATP-diphosphohydrolase activity was stimulated at micromolar

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[120] Toyama Y1, Nakagaki H, Kato S, Huang S, Mizutani Y, Kojima S, Toyama A, Ohno N, Tsuchiya T, Kirkham J, Robinson C. Fluoride concentrations at and near the neonatal line in human deciduous tooth enamel obtained from a naturally fluoridated and a non-fluoridated area. Arch Oral Biol. 2001 Feb;46(2):147-53.

[121] Li Y1, Sun M, Wu D, Chen X. [The toxicity of combination of selenium, fluoride and arsenic on rat embryos]. Wei Sheng Yan Jiu. 1999 Mar 30;28(2):74-6.

[122] Flores-Herrera O1, Uribe A, Pardo JP, Rendón JL, Martínez F. A novel ATP-diphosphohydrolase from human term placental mitochondria. Placenta. 1999 Jul-Aug;20(5-6):475-84.

concentrations of calcium or magnesium in the presence of La-PPi. Negative cooperativity kinetics was observed with all substrates tested. The V(max)ranged from 150 to 300nmol of Pi released/mg/min. The [S](0.5)for nucleotides was 1-10m m and 182m m for PPi. The enzyme was inhibited by orthovanadate, but not by l -phenylalanine, oligomycin, sodium azide, P(1),P(5)-di(adenosine-5')pentaphosphate or sodium fluoride.The experimental evidence showing absence of inhibition by sodium azide and sodium fluoride, hydrolysis of pyrophosphate but not of monoester phosphates, and negative cooperativity suggested that this enzyme was a novel ATP-diphosphohydrolase."

Yuan (1998)[123] "Most inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase function as substrates for the "3'-oxidative activity" of the enzyme and convert the enzyme from its active form (NAD+) to its inactive form (NADH) (Liu, S., Wolfe, M. S., and Borchardt, R. T. (1992) Antivir. Res. 19, 247-265). In this study, we describe the effects of a mechanism-based inhibitor, 6'-bromo-5', 6'-didehydro-6'-deoxy-6'-fluorohomoadenosine (BDDFHA), which functions as a substrate for the "6'-hydrolytic activity" of the enzyme with subsequent formation of a covalent linkage with the enzyme. Incubation of human placental AdoHcy hydrolase with BDDFHA results in a maximum inactivation of 83% with the remaining enzyme activity exhibiting one-third of the kcat value of the native enzyme. This partial inactivation is concomitant with the release of both Br- and F- ions and the formation of adenine (Ade). The enzyme can be covalently labeled with [8-3H]BDDFHA, resulting in a stoichiometry of 2 mol of BDDFHA/mol of the tetrameric enzyme. The 3H-labeled enzyme retains its original NAD+/NADH content. Tryptic digestion and subsequent protein sequencing of the [8-3H]BDDFHA-labeled enzyme revealed that Arg196 is the residue that is associated with the radiolabeled inhibitor. The partition ratio of the Ade formation (nonlethal event) to covalent acylation (lethal event) is approximately 1:1. From these experimental results, a possible mechanism by which BDDFHA inactivates AdoHcy hdyrolase is proposed: enzyme-mediated water addition at the C-6' position of BDDFHA followed by elimination of Br- ion results in the formation of homoAdo 6'-carboxyl fluoride (HACF). HACF then partitions in two ways: (a) attack by a proximal nucleophile (Arg196) to form an amide bond after expulsion of F- ion (lethal event) or (b) depurination to form Ade and hexose-derived 6-carboxyl fluoride (HDCF), which is further hydrolyzed to hexose-derived 6-carboxylic acid (HDCA) and F- ion (nonlethal event). . . . Pharmacological modulation of intracellular methylation can be achieved through feedback inhibition of methyltransferase activity by AdoHcy (2). Intracellular AdoHcy concentrations can be elevated by decreasing AdoHcy hydrolase activity (27). Numerous nucleoside analogs capable of reversibly or irreversibly inhibiting AdoHcy hydrolase have been isolated or synthesized as potential antiviral, antiparasitic, antiarthritic, immunosuppresive, and antitumor agents (3-10). More recently, AdoHcy hydrolase inhibitors have been reported to be specially effective against fliovirus such as Ebola virus (28).

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[123] Yuan CS1, Wnuk SF, Robins MJ, Borchardt RT. A novel mechanism-based inhibitor (6'-bromo-5', 6'-didehydro-6'-deoxy-6'-fluorohomoadenosine) that covalently modifies human placental S-adenosylhomocysteine hydrolase. J Biol Chem. 1998 Jul 17;273(29):18191-7.

Tertrin-Clary (1998)[124] "1. Introduction: Protein kinase C (PKC) plays a fundamental role in the regulation of many signal transduction mechanisms activated in response to a variety of stimuli (hormones, growth factors, neurotransmitters). Molecular cloning and biochemical studies have revealed that this kinase consists of a family of at least 12 closely related isoforms classified into four groups based on their primary structure and cofactor requirements. . . . PKC appears to perform a variety of functions in vascular smooth muscle. Many studies have reported that the activation of PKC is associated with vascular smooth muscle contractility and plays a major role in growth-related signal transduction [2]. The feto-placental circulation provides for the metabolic needs of the fetus, and regulation of blood flow in this system is critical for fetal well-being and normal development. Stem villi vessels are considered to be the major sites of fetal placenta vascular resistance [3]. Since the placental vessels lack autonomic innervation, vascular tone is regulated by locally or humorally delivered vasoactive substances [4]. Endothelin-1 (ET-1), a 21 amino acid peptide, is a potent vasoactive agent that acts on the contractility of placental vessels [5]. Several studies have reported that activation of PKC may be a component of the signal cascade resulting in the effects of this peptide on contractility and cell division in vascular smooth muscles, such as rat cardiomyocytes [6-8], bovine cerebral arteries [9], human and rat renal artery [10,11], rat aorta [12] and the rat portal vein [13]. Specific high affinity binding sites for ET-1 have been described in the muscular layer of stem villi vessels [14], and Mondon et al. [15] demonstrated that these ET-1 vascular binding sites are coupled to a phosphoinositide-specific phospholipase C pathway that generates two intracellular messengers, DAG and CaP^{2+}, that are activators of PKC.

The objective of this study was to examine the presence of PKC activity in the muscular layer of human placental stem villi vessels. . . .

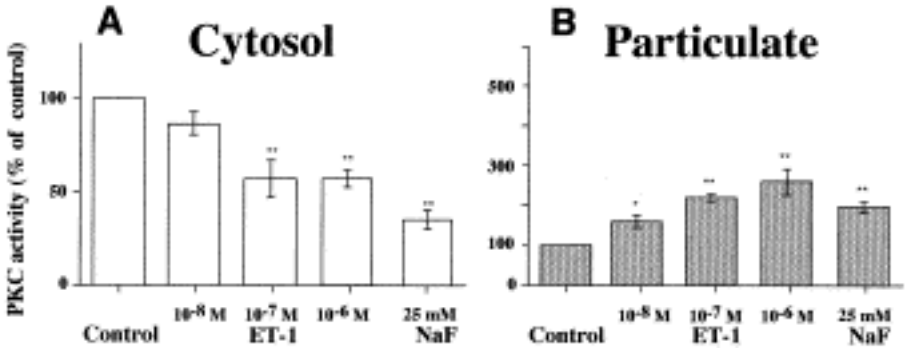

Fig. 1.
Chromatography of cytosolic and particulate-associated protein kinase C from human placental stem villi vessels on a DEAE-cellulose column. PKC activity in the eluted fractions was assayed as described in Section 2and is expressed cpm: (•) in the

[124] Tertrin-Clary C, Fournier T., Ferreè F. Regulation of protein kinase C in the muscular layer of human placental stem villi vessels. FEBS Lett. 1998 Jan 23;422(1):123-8.

presence of Ca^{2+}, phosphatidylserine and diolein, (○) in the presence of EGTA, without phosphatidylserine or diolein. Results are representative of three experiments."

Montherrat-Carret (1996)[125] "To evaluate the beneficial effect of prenatal fluoride supplementation, the presence of fluoride in hard tissues in two populations of human foetuses coming from fluoridated (> or = 0.7 parts/10(6) F in drinking water) and non-fluoridated areas (< or = 0.1 parts/10(6) F in drinking water) were compared by chemical analysis and X-ray microanalysis. The fluoride concentrations measured in maternal and venous cord blood confirmed that placental transfer of fluoride was passive when fluoride intake was low. Total fluoride contents of tooth germs and mandibular bone appeared to increase with fluoride level in drinking water. However, these concentrations were too low to be detected by X-ray microanalysis. Phosphorus and calcium total contents were identical in mandibular and femoral bone of both populations. In incisor germs, phosphorus and calcium concentrations in enamel and dentine close to the amelodentinal junction did not differ significantly between the two populations. It is suggested that the low fluoride concentrations in enamel and dentine formed in utero would not have a significant effect on acid solubility."

Anand (1996)[126] "Active glycine transport was demonstrated in microvillous (maternal-facing, BBM) and basal (fetal-facing, BCM) plasma membranes of the human term placental syncytiotrophoblast. . . Nicotine, insulin, sodium fluoride and sodium arsenate were inhibitors for both the vesicles."

Gupta (1993)[127] "Transplacental passage of fluorides was studied in 25 randomly selected neonates. Blood samples collected simultaneously from the mother and the umbilical cord showed that average fluoride concentration in the cord blood was 60% of that in mother's blood. When concentration in the mother's blood exceeded 0.4 ppm, the placenta acted as a selective barrier.

Malhotra (1993)[128] "The study was conducted on 25 healthy women residing in optimum fluoride areas, who were to deliver normally through vaginal route, to correlate the maternal and cord plasma fluoride levels and evaluate the placental transfer of fluoride. A wide variation was found in the maternal and cord plasma fluoride levels. In only 8 percent of the cases the fluoride levels in cord plasma were higher than maternal plasma. It was deduced that the placenta allows passive diffusion of fluoride from mother to foetus and does not act as a barrier."

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[125]Montherrat-Carret L1, Perrat-Mabilon B, Barbey E, Bouloc R, Boivin G, Michelet A, Magloire H. Chemical and X-ray analysis of fluoride, phosphorus, and calcium in human foetal blood and hard tissues. Arch Oral Biol. 1996 Dec;41(12):1169-78.

[126] Anand RJ1, Kanwar U, Sanyal SN. Transport of glycine in the brush border and basal cell membrane vesicles of the human term placenta. Biochem Mol Biol Int. 1996 Feb;38(1):21-30.

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Vinals (1993)[129] "Fluoride is a nucleophilic reagent which has been reported to inhibit a variety of different enzymes such as esterases, asymmetrical hydrolases and phosphatases. In this report, we demonstrate that fluoride inhibits tyrosine kinase activity of insulin receptors partially purified from rat skeletal muscle and human placenta. . . . These data suggest: (i) that fluoride interacts directly and slowly with the insulin receptor, which causes inhibition of its phosphotransferase activity; (ii) that the binding site of fluoride is not structurally modified by receptor phosphorylation; and (iii) based on the fact that fluoride inhibits phosphotransferase activity in the absence of alterations in the binding of ATP, Mn2+ or insulin, we speculate that fluoride binding might affect the transfer of phosphate from ATP to the tyrosine residues of the beta-subunit of the insulin receptor and to the tyrosine residues of exogenous substrates."

The NRC (2006)[130] concluded in part: "The effects of fluoride on various aspects of endocrine function should be examined further, particularly with respect to a possible role in the development of several diseases or mental states in the United States. Major areas for investigation include the following: . . . thyroid disease (especially in light of decreasing iodine intake by the U.S. population). . . ."

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V. NRC (2006) REPORT ON THE ENDOCRINE SYSTEM

The following 9 pages are directly from pages 224-236 of the NRC's report's "Fluoride in Drinking Water: A Scientific Review of EPA's Standards."

"Effects on the Endocrine System

The endocrine system, apart from reproductive aspects, was not considered in detail in recent major reviews of the health effects of fluoride (PHS 1991; NRC 1993; Locker 1999; McDonagh et al. 2000a; WHO 2002; ATSDR 2003). Both the Public Health Service (PHS 1991) and the World Health Organization (WHO 2002) mentioned secondary hyperparathyroidism in connection with discussions of skeletal fluorosis, but neither report examined endocrine effects any further. The Agency for Toxic Substances and Disease Registry (ATSDR 2003) discussed four papers on thyroid effects and two papers on parathyroid effects and concluded that "there are some data to suggest that fluoride does adversely affect some endocrine glands." McDonagh et al. (2000a) reviewed a number of human studies of fluoride effects, including three that dealt with goiter and one that dealt with age at menarche. The following section reviews material on the effects of fluoride on the endocrine system—in particular, the thyroid (both follicular cells and parafollicular cells), parathyroid, and pineal glands. Each of these sections has its own discussion section. Detailed information about study designs, exposure conditions, and results is provided in Appendix E.

The follicular cells of the thyroid gland produce the classic thyroid hormones thyroxine (T4) and triiodothyronine (T3); these hormones modulate a variety of physiological processes, including but not limited to normal growth and development (Larsen et al. 2002; Larsen and Davies 2002; Goodman 2003). Between 4% and 5% of the U.S. population may be affected by deranged thyroid function (Goodman 2003), making it among the most prevalent of endocrine diseases (Larsen et al. 2002). The prevalence of subclinical thyroid dysfunction in various populations is 1.3-17.5% for subclinical hypothyroidism and 0.6-16% for subclinical hyperthyroidism; the reported rates depend on age, sex, iodine intake, sensitivity of measurements, and definition used (Biondi et

al. 2002). Normal thyroid function requires sufficient intake of iodine (at least 100 micrograms/day [µg/d]), and areas of endemic iodine deficiency are associated with disorders such as endemic goiter and cretinism (Larsen et al. 2002; Larsen and Davies 2002; Goodman 2003). Iodine intake in the United States (where iodine is added to table salt) is decreasing (CDC 2002d; Larsen et al. 2002), and an estimated 12% of the population has low concentrations of urinary iodine (Larsen et al. 2002).

The principal regulator of thyroid function is the pituitary hormone thyroid-stimulating hormone (TSH), which in turn is controlled by positive input from the hypothalamic hormone thyrotropin-releasing hormone (TRH) and by negative input from T4 and T3. TSH binds to G-protein-coupled receptors in the surface membranes of thyroid follicular cells (Goodman 2003), which leads to increases in both the cyclic adenosine monophosphate (cAMP) and diacylglycerol/inositol trisphosphate second messenger pathways (Goodman 2003). T3, rather than T4, probably is responsible for the feedback response for TSH production (Schneider et al. 2001). Some T3, the active form of thyroid hormone, is secreted directly by the thyroid along with T4, but most T3 is produced from T4 by one of two deiodinases (Types I and II1) in the peripheral tissue (Schneider et al. 2001; Larsen et al. 2002; Goodman 2003). T3 enters the nucleus of the target cells and binds to specific receptors, which activate specific genes.

Background

An effect of fluoride exposure on the thyroid was first reported approximately 150 years ago (Maumené 1854, 1866; as cited in various reports). In 1923, the director of the Idaho Public Health Service, in a letter to the Surgeon General, reported enlarged thyroids in many children between the ages of 12 and 15 using city water in the village of Oakley, Idaho (Almond 1923); in addition, the children using city water had severe enamel deficiencies in their permanent teeth. The dental problems were eventually attributed to the presence in the city water of 6 mg/L fluoride, and children born after a change in water supply (to water with <0.5 mg/L fluoride) were not so affected (McKay 1933); however, there seems to have been no further report on thyroid conditions in the village.

More recently, Demole (1970) argued that a specific toxicity of fluoride for the thyroid gland does not exist, because (1) fluoride does not accumulate in the thyroid; (2) fluoride does not affect the uptake of iodine by thyroid tissue; (3) pathologic changes in the thyroid show no increased frequency in regions where water is fluoridated (naturally or artificially); (4) administration of fluoride does not interfere with the prophylactic action of iodine on endemic goiter; and (5) the beneficial effect of iodine in threshold dosage to experimental animals is not inhibited by administration of fluoride, even in excessive amounts. Bürgi et al. (1984) also stated that fluoride does not potentiate the consequences of iodine deficiency in populations with a borderline or low iodine intake and that published data fail to support the hypothesis that fluoride has adverse effects on the thyroid (at doses recommended for caries prevention). McLaren (1976), however, pointed out the complexity of the system, the difficulties in making adequate comparisons of the various studies of fluoride and the thyroid, and evidence for fluoride accumulation in the thyroid and morphological and functional changes (e.g., changes in activity of adenylyl cyclase), suggesting that analytical methods could have limited the

definitiveness of the data to date. His review suggested that physiological or functional changes might occur at fluoride intakes of 5 mg/day.

Although fluoride does not accumulate significantly in most soft tissue (as compared to bones and teeth), several older studies found that fluoride concentrations in thyroid tissue generally exceed those in most other tissue except kidney (e.g., Chang et al. 1934; Hein et al. 1954, 1956); more recent information with improved analytic methods for fluoride was not located. Several studies have reported no effect of fluoride treatment on thyroid weight or morphology (Gedalia et al. 1960; Stolc and Podoba 1960; Saka et al. 1965; Bobek et al. 1976; Hara 1980), while others have reported such morphological changes as mild atrophy of the follicular epithelium (Ogilvie 1953), distended endoplasmic reticulum in follicular cells (Sundström 1971), and "morphological changes suggesting hormonal hypofunction" (Jonderko et al. 1983).

Fluoride was once thought to compete with iodide for transport into the thyroid, but several studies have demonstrated that this does not occur (Harris and Hayes 1955; Levi and Silberstein 1955; Anbar et al. 1959; Saka et al. 1965). The iodide transporter accepts other negatively charged ions besides iodide (e.g., perchlorate), but they are about the same size as iodide (Anbar et al. 1959); fluoride ion is considerably smaller and does not appear to displace iodide in the transporter.

Animal Studies

A number of studies have examined the effects of fluoride on thyroid function in experimental animals or livestock (for details, see Appendix E, Tables E-1, E-2, and E-3). Of these, the most informative are those that have considered both the fluoride and iodine intakes.

Guan et al. (1988) found that a fluoride intake of 10 mg/L in drinking water had little apparent effect on Wistar rats with sufficient iodine intake, but a fluoride intake of 30 mg/L in drinking water resulted in significant decreases in thyroid function (decreases in T4, T3, thyroid peroxidase, and 3H-leucine), as well as a decrease in thyroid weight and effects on thyroid morphology (Table E-2). In iodine-deficient rats, fluoride intake of 10 mg/L in drinking water produced abnormalities in thyroid function beyond that attributable to low iodine, including decreased thyroid peroxidase, and low T4 without compensatory transformation of T4 to T3.

Zhao et al. (1998), using male Kunmin mice, found that both iodine-deficient and iodine-excess conditions produced goiters, but, under iodine-deficient conditions, the goiter incidence at 100 days increased with increased intake of fluoride. At 100 days, the high-fluoride groups had elevated serum T4 at all concentrations of iodine intake and elevated T3 in iodine-deficient animals. High fluoride intake significantly inhibited the radioiodine uptake in the low- and normal-iodine groups.

Stolc and Podoba (1960) found a decrease in protein-bound iodine in blood in fluoride-treated female rats (3-4 mg/kg/day) fed a low-iodine diet but not in corresponding rats fed a larger amount of iodine. Both groups (low- and high-iodine) of fluoride-treated rats showed a reduced rate of biogenesis of T3 and T4 after administration of 131I compared with controls (Stolc and Podoba 1960).

Bobek et al. (1976) found decreases in plasma T4 and T3 as well as a decrease in free T4 index and an increase in T3-resin uptake in male rats given 0.1 or 1 mg of fluoride per day (0.4-0.6 or 4-6 mg/kg/day) in drinking water for 60 days.2 The authors suggested the possibility of decreased binding capabilities and altered thyroid hormone transport in blood.

Decreases in T4 and T3 concentrations have been reported in dairy cows at estimated fluoride doses up to 0.7 mg/kg/day with possible iodine deficiency (Hillman et al. 1979; Table E-3). Reduced T3 (Swarup et al. 1998) and reduced T3, T4, and protein-bound iodine (Cinar and Selcuk 2005) have also been reported in cows diagnosed with chronic fluorosis in India and Turkey, respectively.

Hara (1980) found elevated T3 and T4 at the lowest dose (approximately 0.1 mg/kg/day), decreased T3 and normal T4 at intermediate doses (3-4 mg/kg/day), and decreased TSH and growth hormone (indicating possible effects on pituitary function) at the highest doses (10-20 mg/kg/day). This was the only animal study of fluoride effects on thyroid function to measure TSH concentrations; however, full details (e.g., iodine intake) are not available in English.

Other studies have shown no effect of fluoride on the end points examined (Gedalia et al. 1960; Siebenhüner et al. 1984; Clay and Suttie 1987; Choubisa 1999; Table E-1). Choubisa (1999) looked only for clinical evidence of goiter in domestic animals (cattle and buffaloes) showing signs of enamel or skeletal fluorosis; no hormone parameters (e.g., T4, T3, TSH) were measured. Gedalia et al. (1960) also did not measure T4, T3, or TSH; radioiodine uptake, protein-bound iodine, and total blood iodine were all normal in rats receiving fluoride doses up to approximately 1 milligram per kilogram of body weight per day (mg/kg/day). Clay and Suttie (1987) reported no significant differences from control values for T4 concentration and T3 uptake in heifers fed up to 1.4 mg/kg/day; iodine intake is not stated but probably was adequate, and TSH was not measured.

Siebenhüner et al. (1984) carried out a special experiment involving iodine depletion of the thyroid before 6 days of fluoride treatment. No effects were seen on the parameters measured, including T3 and T4 concentrations; however, TSH was not measured. In addition, propylthiouracil (PTU), the agent used to deplete the thyroid of iodine, also has an inhibitory effect on deiodinases (Larsen et al. 2002; Larsen and Davies 2002); Siebenhüner et al. (1984) did not mention this second action of PTU and its relevance to the interpretation of the experimental results, and there was no control group without the PTU treatment.

Human Studies

Several authors have reported an association between endemic goiter and fluoride exposure or enamel fluorosis in human populations in India (Wilson 1941; Siddiqui 1960; Desai et al. 1993), Nepal (Day and Powell-Jackson 1972), England (Wilson 1941; Murray et al. 1948), South Africa (Steyn 1948; Steyn et al. 1955; Jooste et al. 1999), and Kenya (Obel 1982). Although endemic goiter is now generally attributed to iodine deficiency (Murray et al. 1948; Obel 1982; Larsen et al. 2002; Belchetz and Hammond 2003), some of the goitrogenic areas associated with fluoride exposure were not considered to be iodine deficient (Steyn 1948; Steyn et al. 1955; Obel 1982; Jooste et

al. 1999). Obel (1982) indicated that many cases of fluorosis in Kenya occur concurrently with goiter. Several authors raise the possibility that the goitrous effect, if not due to fluoride, is due to some other substance in the water (e.g., calcium or water hardness) that was associated with the fluoride concentration (Murray et al. 1948; Day and Powell-Jackson 1972) or that enhanced the effect of fluoride (Steyn 1948; Steyn et al. 1955). Dietary selenium deficiencies (e.g., endemic in parts of China and Africa or due to protein-restricted diets) can also affect normal thyroid function3 (Larsen et al. 2002); no information on dietary selenium is available in any of the fluoride studies. Appendix E summarizes a number of studies of the effects of fluoride on thyroid function in humans (see Table E-4).

Three studies illustrated the range of results that have been reported: (1) Gedalia and Brand (1963) found an association between endemic goiter in Israeli girls and iodine concentrations in water but found no association with fluoride concentrations (<0.1-0.9 mg/L). (2) Siddiqui (1960) found goiters only in persons aged 14-17 years; the goiters, which became less visible or invisible after puberty, were associated with mean fluorine content of the water (5.4-10.7 mg/L) and were inversely associated with mean iodine content of the water. (3) Desai et al. (1993) found a positive correlation ($P < 0.001$) between prevalence of goiter (9.5-37.5%) and enamel fluorosis (6.0-59.0%), but no correlation between prevalence of goiter and water iodine concentration ($P > 0.05$).

Day and Powell Jackson (1972) surveyed 13 villages in Nepal where the water supply was uniformly low in iodine (?1 µg/L; see Figure 8-1). Here the goiter prevalence (5-69%, all age groups) was directly associated with the fluoride concentration (<0.1 to 0.36 mg/L; $P < 0.01$) or with hardness, calcium concentration, or magnesium concentration of the water (all $P < 0.01$). Goiter prevalence of at least 20% was associated with all fluoride concentrations ? 0.19 mg/L, suggesting that fluoride might influence the prevalence of goiter in an area where goiter is endemic because of low iodine intake. The possibility of a nutritional component (undernutrition or protein deficiency) to the development of goiter was also suggested.

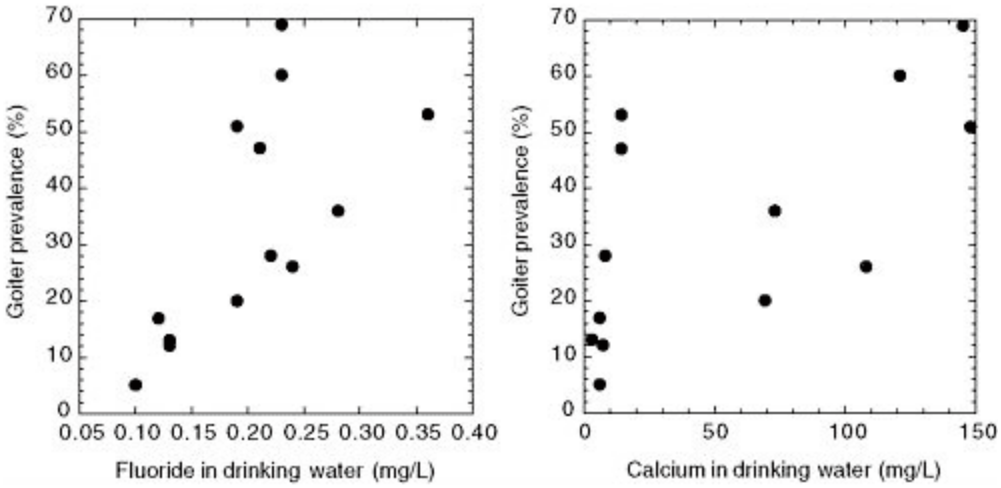
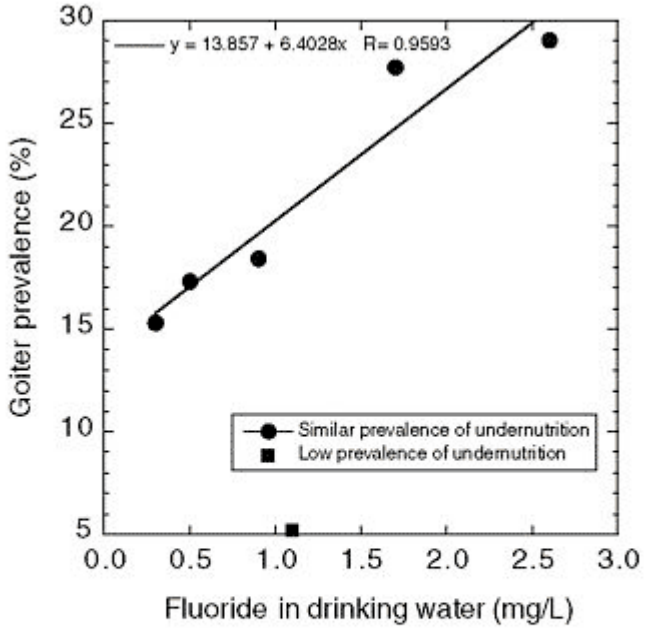


FIGURE 8-1 Goiter prevalence versus fluoride (left) and calcium (right) concentration in drinking water for 13 villages in Nepal with very low iodine concentrations. SOURCE: Day and Powell-Jackson 1972.

Jooste et al. (1999) examined children (ages 6, 12, and 15) who had spent their entire lives in one of six towns in South Africa where iodine concentrations in drinking water were considered adequate (median urinary iodine concentration exceeding 201 µg/L [1.58 µmol/L]; see Appendix E, Tables E-4 and E-5; Figure 8-2). For towns with low (0.3-0.5 mg/L) or near "optimal" (0.9-1.1 mg/L) fluoride concentrations in water, no relationship between fluoride and prevalence of mild goiter was found (5-18%); for the other two towns (1.7 and 2.6 mg/L fluoride), however, goiter prevalences were 28% and 29%, respectively, and most children had severe enamel mottling. These two towns (and one low-fluoride town) had very low proportions (0-2.2%) of children with iodine deficiency, defined as urinary iodine concentrations <100 µg/L (<0.79 µmol/L). The town with the lowest prevalence of goiter also had the lowest prevalence of under-nutrition; the two towns with the highest prevalence of goiter (and highest fluoride concentrations) did not differ greatly from the remaining three towns with respect to prevalence of under-nutrition. The authors suggested that fluoride or an associated goitrogen might be responsible for the goiters seen in the two towns with the highest fluoride concentrations but that some other factor(s) was involved in development of goiter in the other towns.



Several studies have compared various aspects of thyroid status in populations with different fluoride intakes (for details, see Appendix E, Table E-4). Leone et al. (1964) and Baum et al. (1981) reported no significant differences in thyroid status between populations with low (0.09-0.2 mg/L) and high (3-3.5 mg/L) fluoride concentrations

in the drinking water. Leone et al. (1964) looked only at protein-bound iodine and physical examination of the thyroid in adults; Baum et al. (1981) measured a number of parameters in teenagers, including T4, T3, and TSH. Neither study reported iodine status of the groups. Baum et al. (1981) showed but did not explain a decrease in thyroglobulin in girls in the high-fluoride group.

Bachinskii et al. (1985) examined 47 healthy persons, 43 persons with hyperthyroidism, and 33 persons with hypothyroidism. Prolonged consumption of “high-fluoride” drinking water (2.3 mg/L, as opposed to “normal” concentrations of 1 mg/L) by healthy persons was associated with statistically significant changes in TSH concentrations (increased), T3 concentrations (decreased), and uptake of radioiodine (increased), although the mean values for TSH and T3 were still within normal ranges (see Appendix E, Table E-6). The mean value of TSH for the healthy group (4.3 ± 0.6 milliunits/L; Table E-6) is high enough that one expects a few individuals to have been above the normal range (typically 0.5-5 milliunits/L; Larsen et al. 2002). These results were interpreted as indicating disruption of iodine metabolism, stress in the pituitary-thyroid system, and increased risk of developing thyroidopathy (Bachinskii et al. 1985).

Lin et al. (1991) examined 769 children (7-14 years old) for mental retardation in three areas of China, including an area with “high” fluoride (0.88 mg/L) and low iodine, an area with “normal” fluoride (0.34 mg/L) and low iodine, and an area where iodine supplementation was routine (fluoride concentration not stated). Ten to twelve children in each area received detailed examinations, including measuring thyroid 131I uptake and thyroid hormone concentrations. Children in the first area had higher TSH, slightly higher 131I uptake, and lower mean IQ than children in the second area. Children in the first area also had reduced T3 and elevated reverse T3, compared with children in the second area. The authors suggested that high fluoride might exacerbate the effects of iodine deficiency. In addition, the authors reported a difference in T3/rT3 (T3/reverse-T3) ratios between high- and low-fluoride areas and suggested that excess fluoride ion affects normal deiodination.

A recent study by Susheela et al. (2005) compared thyroid hormone status (free T4, free T3, and TSH) of 90 children with enamel fluorosis (drinking water fluoride ranging from 1.1 to 14.3 mg/L) and 21 children without enamel fluorosis (0.14-0.81 mg/L fluoride in drinking water) in areas where iodine supplementation was considered adequate.4 Forty-nine children (54.4%) in the sample group had “well-defined hormonal derangements”; findings were borderline in the remaining 41 children. The types of hormonal derangements included elevated TSH and normal T4 and T3 (subclinical hypothyroidism); low T3 and normal T4 and TSH (“low T3 syndrome”); elevated T3 and TSH and normal T4 (possible T3 toxicosis); elevated TSH, low T4, and normal T3 (usually indicative of primary hypothyroidism and iodine deficiency); and low T3, high TSH, and normal T4. All but the first category are considered to be associated with or potentially caused by abnormal activity of deiodinases. The authors concluded that fluoride in excess may be inducing diseases that have usually been attributed to iodine deficiency and that iodine supplementation may not be adequate when excess fluoride is being consumed.

Thyroid hormone disturbances were also noted in the control children, and urine and fluoride concentrations in the control children reflect higher fluoride intake than can be

accounted for by the drinking water alone (Susheela et al. 2005). Thus, the authors recommend that end points such as hormone concentrations should be examined with respect to serum or urinary fluoride concentrations, not just drinking water fluoride concentrations. In addition, they note that all hormone endpoints (T3, T4, and TSH) should be examined, lest some of the abnormalities be missed.

Mikhailets et al. (1996) detected thyroid abnormalities (moderate reduction of iodine uptake, low T3, normal T4, and increased TSH) in 165 aluminum workers with signs of chronic fluorosis and an estimated average fluoride intake of 10 mg/working day. A tendency toward increased TSH was observed with increased exposure time and with more severe fluorosis. Workers with more than 10 years of service had a significant decrease in T3 concentration in comparison to controls. The frequency of individuals with low concentrations of T3 (corresponding to hypothyroidism) was 65% among workers with more than 10 years of service and 54% among workers with Stage 2 fluorosis. The highest frequency of occurrence of low T3 (76%) was observed in people with chronic fluoride intoxication including liver damage (moderate cytolysis), suggesting a disorder in peripheral conversion of T4 to T3 (deiodination). The possibility of indirect effects of fluorine on enzymatic deiodination was also suggested.

Tokar? et al. (1989) and Balabolkin et al. (1995) have also reported thyroid effects in fluoride- or fluorine-exposed workers; full details of these studies are not available in English. Balabolkin et al. (1995) found that 51% of the workers examined had subclinical hypothyroidism with reduced T3.

No changes in thyroid function were detected in two studies of osteoporosis patients treated with NaF for 6 months or several years (Eichner et al. 1981; Hasling et al. 1987; for details, see Appendix E, Table E-7). These study populations are not necessarily representative of the general population, especially with respect to age and the fact that they usually receive calcium supplements. In an earlier clinical study to examine the reported effects of fluoride on individuals with hyperthyroidism, Galletti and Joyet (1958) found that, in 6 of 15 patients, both basal metabolic rate and protein-bound iodine fell to normal concentrations, and the symptoms of hyperthyroidism were relieved after fluoride treatment. Fluoride was considered clinically ineffective in the other 9 patients, although improvement in basal metabolic rate or protein-bound iodine was observed in some of them. In the 6 patients for whom fluoride was effective, tachycardia and tremor disappeared within 4-8 weeks, and weight loss was stopped. The greatest clinical improvement was observed in women between 40 and 60 years old with a moderate degree of thyrotoxicosis; young patients with the classic symptoms of Graves' disease did not respond to fluoride therapy. Radioiodine uptake tests were performed on 10 of the patients, 7 of whom showed an inhibitory effect on initial 131I uptake by the thyroid.

Discussion (Effects on Thyroid Function)

In studies of animals with dietary iodine sufficiency, effects on thyroid function were seen at fluoride doses of 3-6 mg/kg/day (Stolc and Podoba 1960; Bobek et al. 1976; Guan et al. 1988; Zhao et al. 1998); in one study, effects were seen at doses as low as 0.4-0.6 mg/kg/day (Bobek et al. 1976). In low-iodine situations, more severe effects on thyroid function were seen at these doses (Stolc and Podoba 1960; Guan et al. 1988; Zhao et al. 1998). Effects on thyroid function in low-iodine situations have also been noted at fluoride doses as low as 0.06 mg/kg/day (Zhao et al. 1998), ?0.7 mg/kg/day

(Hillman et al. 1979), and 1 mg/kg/day (Guan et al. 1988). Studies showing no effect of fluoride on thyroid function did not measure actual hormone concentrations (Gedalia et al. 1960; Choubisa 1999), did not report iodine intakes (Gedalia et al. 1960; Clay and Suttie 1987; Choubisa 1999), used fluoride doses (<1.5 mg/kg/day) below those (3-6 mg/kg/day) associated with effects in other studies (Gedalia et al. 1960; Clay and Suttie 1987), or did not discuss a possibly complicating factor of the experimental procedure used (Siebenhüner et al. 1984). Only one animal study (Hara 1980) measured TSH concentrations, although that is considered a “precise and specific barometer” of thyroid status in most situations (Larsen et al. 2002). Full details of Hara’s report are not available in English.

Goiter prevalence of at least 20% has been reported in humans exposed to water fluoride concentrations ? 0.2 mg/L (low-iodine situation; Day and Powell-Jackson 1972) or 1.5-3 mg/L (undernutrition, but adequate iodine; Jooste et al. 1999); however, other causes of goiter have not been ruled out. Bachinskii et al. (1985) showed increased TSH concentrations and reduced T3 concentrations in a population with a fluoride concentration of 2.3 mg/L in their drinking water (in comparison to a group with 1.0 mg/L), and Lin et al. (1991) showed similar results for a population with 0.88 mg/L fluoride in the drinking water (in comparison to a group with 0.34 mg/L); another study showed no effect at 3 mg/L (Baum et al. 1981). Among children considered to have adequate iodine supplementation, Susheela et al. (2005) found derangements of thyroid hormones in 54% of children with enamel fluorosis (1.1-14.3 mg/L fluoride in drinking water), and in 45-50% of “control” children without enamel fluorosis but with elevated serum fluoride concentrations. Mikhailets et al. (1996) observed an increase in TSH in workers with increased exposure time and with more severe fluorosis; low T3 was found in 65% of workers with more than 10 years of service and in 54% of workers with Stage 2 fluorosis. Several studies do not include measurements of T4, T3, or TSH (Siddiqui 1960; Gedalia and Brand 1963; Leone et al. 1964; Day and Powell-Jackson 1972; Teotia et al. 1978; Desai et al. 1993; Jooste et al. 1999).

Nutritional information (especially the adequacy of iodine and selenium intake) is lacking for many (iodine) or all (selenium) of the available studies on humans. As with the animal studies, high fluoride intake appears to exacerbate the effects of low iodine concentrations (Day and Powell-Jackson 1972; Lin et al. 1991). Uncertainty about total fluoride exposures based on water fluoride concentrations, variability in exposures within population groups, and variability in response among individuals generally have not been addressed. Although no thyroid effects were reported in studies using controlled doses of fluoride for osteoporosis therapy, the study populations are not necessarily representative of the general population with respect to age, calcium intake, and the presence of metabolic bone disease.

Thus, several lines of information indicate an effect of fluoride exposure on thyroid function. However, because of the complexity of interpretation of various parameters of thyroid function (Larsen et al. 2002), the possibility of peripheral effects on thyroid function instead of or in addition to direct effects on the thyroid, the absence of TSH measurements in most of the animal studies, the difficulties of exposure estimation in human studies, and the lack of information in most studies on nutritional factors (iodine, selenium) that are known to affect thyroid function, it is difficult to predict exactly what

effects on thyroid function are likely at what concentration of fluoride exposure and under what circumstances.

Suggested mechanisms of action for the results reported to date include decreased production of thyroid hormone, effects on thyroid hormone transport in blood, and effects on peripheral conversion of T4 to T3 or on normal deiodination processes, but details remain uncertain. Both peripheral conversion of T4 to T3 and normal deiodination (deactivation) processes require the deiodinases (Types I and II for converting T4 to T3 and Types I and III for deactivation; Schneider et al. 2001; Larsen et al. 2002; Goodman 2003). Several sets of reported results are consistent with an inhibiting effect of fluoride on deiodinase activity; these effects include decreased plasma T3 with normal or elevated T4 and TSH and normal T3 with elevated T4 (Bachinskii et al. 1985; Guan et al. 1988; Lin et al. 1991; Balabolkin et al. 1995; Michael et al. 1996; Mikhailets et al. 1996; Susheela et al. 2005). The antihyperthyroid effect that Galletti and Joyet (1958) observed in some patients is also consistent with an inhibition of deiodinase activity in those individuals.

The available studies have generally dealt with mean values of various parameters for the study groups, rather than with indications of the clinical significance, such as the fraction of individuals with a value (e.g., TSH concentration) outside the normal range or with clinical thyroid disease. For example, in the two populations of asymptomatic individuals compared by Bachinskii et al. (1985), the elevated mean TSH value in the higher-fluoride group is still within the normal range, but the number of individuals in that group with TSH values above the normal range is not given.

In the absence of specific information in the reports, it cannot be assumed that all individuals with elevated TSH or altered thyroid hormone concentrations were asymptomatic, although many might have been. For asymptomatic individuals, the significance of elevated TSH or altered thyroid hormone concentrations is not clear. Belchetz and Hammond (2003) point out that the population-derived reference standards (e.g., for T4 and TSH) reflect the mean plus or minus two standard deviations, meaning that 5% of normal people have results outside a given range. At the same time, healthy individuals might regulate plasma T4 within a “personal band” that could be much more narrow than the reference range; this brings up the question of whether a disorder shifting hormone values outside the personal band but within the population reference range requires treatment (Davies and Larsen 2002; Belchetz and Hammond 2003). For example, early hypothyroidism can present with symptoms and raised TSH but with T4 concentrations still within the reference range (Larsen et al. 2002; Belchetz and Hammond 2003).

Subclinical hypothyroidism is considered a strong risk factor for later development of overt hypothyroidism (Weetman 1997; Helfand 2004). Biondi et al. (2002) associate subclinical thyroid dysfunction (either hypo or hyperthyroidism) with changes in cardiac function and corresponding increased risks of heart disease. Subclinical hyperthyroidism can cause bone demineralization, especially in postmenopausal women, while subclinical hypothyroidism is associated with increased cholesterol concentrations, increased incidence of depression, diminished response to standard psychiatric treatment, cognitive dysfunction, and, in pregnant women, decreased IQ of their offspring (Gold et al. 1981; Brucker-Davis et al. 2001). Klein et al. (2001) report an

inverse correlation between severity of maternal hypothyroidism (subclinical or asymptomatic) and the IQ of the offspring (see also Chapter 7).

A number of authors have reported delayed eruption of teeth, enamel defects, or both, in cases of congenital or juvenile hypothyroidism (Hinrichs 1966; Silverman 1971; Biggerstaff and Rose 1979; Noren and Alm 1983; Loevy et al. 1987; Bhat and Nelson 1989; Mg'ang'a and Chindia 1990; Pirinen 1995; Larsen and Davies 2002; Hirayama et al. 2003; Ionescu et al. 2004). No information was located on enamel defects or effects on eruption of teeth in children with either mild or subclinical hypothyroidism. The possibility that either dental fluorosis (Chapter 4) or the delayed tooth eruption noted with high fluoride intake (Chapter 4; see also Short 1944) may be attributable at least in part to an effect of fluoride on thyroid function has not been studied." (End quote of NRC)

VI. FLUORIDE, IODINE AND GOITER[131]

A reasonably consistent body of animal and human research shows that fluoride exposure worsens the impact of iodine deficiency. (Gas'kov 2005; Hong 2001; Wang

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131 Additional References

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- 41:336-339).Yao Y, et al. (1996). Analysis on TSH and intelligence level of children with dental Fluorosis in a high fluoride area. *Literature and Information on Preventive Medicine* 2(1):26-27.
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2001; Zhao 1998; Xu 1994; Lin 1991; Ren 1989; Guan 1988).[132] Iodine is needed for T3 and T4 hormone production and thus an adequate iodine intake is considered important for the proper thyroid function.

Researchers report an iodine deficiency coupled with fluoride exposure produces a more damaging effect on neurological development than iodine deficiency alone. (Hong 2001; Xu 1994; Lin 1991; Ren 1989).[133] The studies, which utilize childhood intelligence as the metric for assessing neurological health, have found that fluoride levels in water as low as 0.9 ppm can worsen the IQ effect of iodine deficiency. (Lin 1991).[134] Studies have reported an association between fluoride and reduced IQ among children with adequate iodine intake, (Choi 2012),[135] and iodine deficiency appears to lower the threshold at which fluoride damages the brain, (Xu 1994; Guan 1988).[136] and dental fluorosis. (Zhao 1998; see also Pontigo-Loyola 2008).[137]

Iodine deficiency is still a public health concern in the United States. (CDC 1998). More than 11% of all Americans, and more than 15% of American women of child-bearing age, presently have urine iodine levels less than 50 mcg/L (Caldwell et al., 2008),[138] indicating moderate to severe iodine deficiency. An additional 36% of reproductive-aged women in the U.S. are considered mildly iodine deficient (<100 mcg/L urinary iodine). Without success, the National Research Council has therefore called for studies investigating the interactive effects of fluoride and iodine on US populations.

The Fluoride Goiter Iodine Connection

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[132] Gas'kov A, et al. (2005). The specific features of the development of iodine deficiencies in children living under environmental pollution with fluorine compounds. *Gig Sanit.* Nov-Dec;(6):53-5.
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Wang X, et al. (2001). Effects of high iodine and high fluorine on children's intelligence and thyroid function. *Chinese Journal of Endemiology* 20(4):288-90.
Xu Y, et al. (1994). The effect of fluorine on the level of intelligence in children. *Endemic Diseases Bulletin* 9(2):83-84.
Lin F, et al (1991). The relationship of a low-iodine and high-fluoride environment to subclinical cretinism in Xinjiang. *Endemic Disease Bulletin* 6(2):62-67 (republished in *Iodine Deficiency Disorder Newsletter* Vol. 7(3):24-25).
Ren D, et al. (1989). A study of the intellectual ability of 8-14 year-old children in high fluoride, low iodine areas. *Chinese Journal of Control of Endemic Diseases* 4:251.
Guan ZZ, et al. (1988). Synergistic action of iodine-deficiency and fluorine-intoxication on rat thyroid. *Chinese Medical Journal* 101(9):679-84.

[133] Ibid #6.

[134] Ibid #6

[135] Choi AL, et al. (2012). Developmental Fluoride Neurotoxicity: A Systematic Review and Meta-Analysis. *Environmental Health Perspectives* 2012 Jul 20. [Epub ahead of print]

[136] Ibid #6

[137] Zhao W, Zhu H, Yu Z, Aoki K, Misumi J, Zhang X. 1998. Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. *Endocrine Regulation* 32(2):63-70.
Pontigo-Loyola A, et al. (2008). Dental fluorosis in 12- and 15-year-olds at high altitudes in above-optimal fluoridated communities in Mexico. *Journal of Public Health Dentistry* 68(3):163-66.

[138] Caldwell KL, et al. (2008). Iodine status of the U.S. population, National Health and Nutrition Examination Survey 2003-2004. *Thyroid* 18(11):1207-14.

Studies dating back to the 19th century have implicated fluoride as a possible cause of goitre. Goitre (aka goiter) is an enlargement of the thyroid gland that in some cases can produce visible swelling in the neck. Although the main cause of goitre is iodine deficiency, it can also be caused by other things, including hypothyroidism and goitrogens (substances that cause goitre). Studies that have examined human populations with adequate intake of iodine have reported mixed results about fluoride's ability to produce goitre. (NRC 2006; Burgi 1984; McLaren 1969).[139] The research has been more consistent, however, where the examined populations had either excessive iodine intakes, or deficient iodine intakes. (Gas'kov 2005; Hong 2001; Wang 2001; Xu 1994; Yang 1994; Lin 1986).[140] Most of this latter research was initially published in either Russian or Chinese and was only recently translated into English by the Fluoride Action Network. Accordingly, previous reviews of fluoride/goitre research (e.g., NRC 2006) were not able to take these studies into account. As such, the evidence linking fluoride to goitre for populations with excessive, or deficient, iodine exposure is stronger than previously recognized.

Dogs have been found to suffer a high incidence of hypothyroidism, the relationship between fluoride contamination and thyroid disease in pets deserves further attention, particularly since it was fluoride's production of goiter in dogs that first prompted the idea that fluoride could be an anti-thyroid agent. (Maumene 1854).[141]

A consistent body of animal and human research shows that fluoride exposure worsens the impact of an iodine deficiency. Iodine is the basic building block of the T3 and T4 hormones and thus an adequate iodine intake is essential for the proper functioning of the thyroid gland. When iodine intake is inadequate during infancy and early childhood, the child's brain can suffer permanent damage, including mental retardation.[142]

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[139] Burgi H, et al. (1984). Fluorine and the Thyroid Gland: A Review of the Literature. *Klin Wochenschr*. 1984 Jun 15;62(12):564-9.
National Research Council. (2006). Fluoride in drinking water: a scientific review of EPA's standards. National Academies Press, Washington D.C.

[140] See Footnote #6
Yang Y, et al. (1994). The effects of high levels of fluoride and iodine on intellectual ability and the metabolism of fluoride and iodine. *Chinese Journal of Epidemiology* 15(4):296-98 (republished in *Fluoride* 2008;
Lin F, et al. (1986). A preliminary approach to the relationship of both endemic goiter and fluorosis in the valley of Manasi

[141] Maumené E. (1854). Experiencé pour déterminer l'action des fluores sur l'economie animale. *Compt Rend Acad Sci (Paris)* 39:538-539.

[142] See previous Nomination to OHAT for Fluoride and Neurological development.
See also

- Ge Y, et al. (2011). Proteomic analysis of brain proteins of rats exposed to high fluoride and low iodine. Archives of Toxicology 85(1):27-33.
- Ge Y, et al. (2005a). Comet assay of DNA damage in brain cells of adult rats exposed to high fluoride and low iodine. Fluoride 38(3):209-14.
- Ge Y, et al. (2005b). DNA damage in thyroid gland cells of rats exposed to long-term intake of high fluoride and low iodine. Fluoride 38(4): 318-323.
- Shen X, Zhang Z, Xu X. (2004). [Influence of combined iodine and fluoride on phospholipid and fatty acid composition in brain cells of rats] Wei Sheng Yan Jiu. 33(2):158-61.
- Wang J, Ge Y, Ning H, Wang S. (2004). Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. Fluoride 37(4): 201-208.

In China, researchers have repeatedly found that an iodine deficiency coupled with fluoride exposure produces a significantly more damaging effect on neurological development than iodine deficiency alone. In the first study to investigate the issue,

Ren (1989) "From the results it is evident that disrupted child intellectual development is among the effects on the human body from a harmful environment containing both high fluoride and low iodine, and this disruption is clearly much more serious than the effects of iodine deficiency alone."[143]

In 1991, a UNICEF-funded study concluded that fluoride levels of just 0.9 ppm (less than the level added to many water supplies for fluoridation) were sufficient to worsen the effects of iodine deficiency. The authors found that, when compared to children with iodine deficiency in a low-fluoride area, the children with iodine deficiency in the 0.9 ppm area had increased TSH levels, reduced T3, reduced intelligence, retarded bone development, and reduced hearing. According to the authors:

"Statistically significant differences existed between these areas, suggesting that a low iodine intake coupled with high fluoride intake exacerbates the central nervous lesions and the somatic developmental disturbance of iodine deficiency."[144]

In 1994, Xu and colleagues measured the IQ rates of children living in 8 areas with differing levels of both iodine and fluoride in exposure. Of all the areas studied, the region with the high fluoride/low iodine content had the lowest IQ. In addition, when compared against the low-iodine area, the high fluoride/low iodine area had a significantly higher rate of thyroid swelling. According to the authors:
"A higher chance of one being affected by thyroid swelling is likewise more prevalent in regions containing a high amount of fluoride but low amount of iodine, and regions where a relatively lower amount of iodine is detected. We believe that in a region where the level of iodine is low, but fluoride is significantly elevated, the level of toxicity in thyroid swelling could increase."[145]

Wang (2004) "In comparison with control rats, the learning and memory ability of the offspring rats was depressed by high fluoride, low iodine, or the combination of high fluoride and low iodine. Brain protein was decreased by low iodine and even more by the combined interaction of high fluoride and low iodine. The activity of cholinesterase (ChE) in the brain was affected to some extent by high fluoride and low iodine but was especially affected by high fluoride and low iodine together."[146]

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[143] Ren D, et al. (1989). A study of the intellectual ability of 8-14 year-old children in high fluoride, low iodine areas. Chinese Journal of Control of Endemic Diseases 4(4):251 (republished in Fluoride 2008; 41:319-20).

[144] SOURCE: Lin Fa-Fu; et al (1991). The relationship of a low-iodine and high-fluoride environment to subclinical cretinism in Xinjiang. Endemic Disease Bulletin 6(2):62-67 (republished in Iodine Deficiency Disorder Newsletter Vol. 7(3):24-25).

[145] Xu Y, et al. (1994). The effect of fluorine on the level of intelligence in children. *Endemic Disease Bulletin* 9(2):83-84.

[146] Wang J, et al. (2004). Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. Fluoride 37(4): 201-208.

Hong (2001) “The IQ results of this study show no significant difference between the average IQs of those children from the high fluoride only areas and the high fluoride/high iodine areas, however the result from the high fluoride/low iodine group show statistically significant differences as compared to that of the low fluoride/low iodine group.”[147]

The interactive effects of fluoride and low iodine on neurological health is consistent with other research showing that fluoride intensifies the anti-thyroid effects of iodine deficiency, and vice versa.

Guan (1988) “This study reveals that the degree of impairment of thyroid morphology and function is related with the amount of fluorine taken by rats. Goiter occurs in rats with iodine deficiency. Damage to the thyroid is observed in rats on iodine deficient diet and highly fluorinated water [30 ppm]. These changes are much more severe than in rats on a normal level iodine diet and highly fluorinated water. This seems to suggest that competitive antagonistic action exists between fluorine and iodine in the thyroid gland.”[148]

An animal study by Zhao et al (1998) found that fluoride and low iodine have “mutually interacting effects” on the thyroid gland, as evident by changes in thyroid weight, time-specific alterations in thyroid hormone levels, increased bone fluoride content, and increased severity of dental fluorosis. As with other studies, Zhao found that fluoride has interactive effects with iodine excess as well. [See study]

More recently, a team of Russian researchers studied a population with iodine deficiency that was exposed to varying levels of fluoride air pollution. The team found that indices of thyroid disease, including stunted growth and thyroid swelling, were more severe, and prophylactic measures less effective, in the population with heavier exposure to fluoride pollution. According to the authors:
“Natural iodine deficiency and ambient air pollution with fluorine compounds were examined for their combined influence on the prevalence and severity of iodine-deficiency disorders. The excess intake of fluorine was shown to increase the incidence of thyroid diseases and to lower anthropometric indices in children. The preventive measures performed to eliminate iodine-deficiency disorders under intensive ambient air pollution with fluorine compounds were found to be insufficiently effective.”[149]

Fluoride, Low Iodine, and Dental Fluorosis

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[147] Hong F, et al. (2001). Research on the effects of fluoride on child intellectual development under different environments. Chinese Primary Health Care 15(3):56-57 (republished in Fluoride 2008; 41(2):156–60).
[148] Guan ZZ, et al. (1988). Synergistic action of iodine-deficiency and fluorine-intoxication on rat thyroid. Chinese Medical Journal 101(9):679-84.
[149] Gas’kov AIu, et al. (2005). [The specific features of the development of iodine deficiencies in children living under environmental pollution with fluorine compounds]. [Article in Russian] Gig Sanit. 2005 Nov-Dec;(6):53-5.

As noted above, the animal study by Zhao (1998) found that iodine deficiency worsened the severity of dental fluorosis in the fluoride-treated rats. Xu (1994) found far higher rates of dental fluorosis in a population with low iodine exposure, than a similar population with adequate iodine exposure. Although both communities had 0.8 ppm fluoride in the water, the rate of dental fluorosis was 89% in the low-iodine area, which was more than double the fluorosis rate (40%) in the area with adequate iodine. (Similar to dental fluorosis in the USA).

More recently, a research team in Mexico reported a high rate of fluorosis in an area known for iodine deficiency. (Pontigo-Loyola 2008). Since the rate of fluorosis was higher than would be expected under normal circumstances, the authors suggested that iodine deficiency could be one of the factors contributing to the high rate. According to the authors,

"The hypothesized relationship between iodine deficiency and increased prevalence of fluorosis appears to be relevant to Hidalgo."[150]

Iodine Deficiency in the United States

Over the past few decades, the rate of iodine deficiency has increased in the United States. According to the National Research Council (NRC), "Iodine intake in the United States (where iodine is added to table salt) is decreasing, and an estimated 12% of the population has low concentrations of urinary iodine." (NRC 2006). In light of this trend, the NRC has called upon researchers to begin studying the endocrine and neurological effects that fluoride exposures may be having on the health of people with low iodine intake. As the NRC stated in 2006:

"The effects of fluoride on various aspects of endocrine function should be examined further, particularly with respect to a possible role in the development of several diseases or mental states in the United States. Major areas for investigation include the following: thyroid disease (especially in light of decreasing iodine intake by the U.S. population)."

GOITER HISTORY

Goitre (goiter) is an enlargement of the thyroid gland that in some cases can produce visible swelling in the neck. The suggested main deficiency cause of goitre is iodine. Goitre can also be caused by other things, including hypothyroidism and substances that cause goitre (goitrogens).

Since as far back as the 19th century, fluoride has been identified as a possible goitrogen. In the research to date, studies that have examined human populations with adequate intake of iodine have reported mixed results about fluoride's ability to produce

[150]Pontigo-Loyola AP, et al. (2008). Dental fluorosis in 12- and 15-year-olds at high altitudes in above-optimal fluoridated communities in Mexico. Journal of Public Health Dentistry 68(3):163-6.

goitre. (NRC 2006; Burgi 1984; McLaren 1969). Where, however, the examined populations had either excessive iodine intakes, or deficient iodine intakes, the research has been more consistent in finding a goitrogenic effect from fluoride. (Gas'kov 2005; Hong 2001; Wang 2001; Xu 1994; Yang 1994; Lin 1986). Since most of this latter research was initially published in either Russian or Chinese and was only recently translated into English by the Fluoride Action Network, the NRC's review of fluoride's goitrogenic potential (e.g, NRC 2006) was not able to take this evidence into account. As such, the evidence linking fluoride to goitre is stronger than previously determined, at least for populations with excessive, or deficient, exposure to iodine.

Origins of the Fluoride/Goitre Connection:

Fluoride was first suspected to be a goitrogen in 1854, when Maumeme reported producing goitre in a dog after 4 months of daily fluoride exposure (9 to 55 mg/day). Based on this and subsequent research in the early 20th century, doctors in Europe and South America began using fluoride as a medical treatment for hyperthyroidism (over-active thyroids). (McLaren 1969). As a goitrogen, doctors believed fluoride could suppress the thyroid's function and thereby alleviate symptoms in people with overly active thyroids. Subsequent clinical research found merit in this idea, as a daily fluoride treatment of just 2 to 5 mg/day was found capable of reducing thyroid function in a group of hyperthyroid patients. (Galletti & Joyet 1958). Ultimately, however, more effective treatments were discovered and the use of fluoride was phased out by the 1960s. (Merck Index 1968).

Fluoride & Goitre in Humans:

Note: the NRC (2006) review did not include the last decade of research and more studies have been translated.

NRC (2006):

"Three studies illustrated the range of results that have been reported: (1) Gedalia and Brand (1963) found an association between endemic goiter in Israeli girls and iodine concentrations in water but found no association with fluoride concentrations (<0.1-0.9 mg/L). (2) Siddiqui (1960) found goiters only in persons aged 14-17 years; the goiters, which became less visible or invisible after puberty, were associated with mean fluorine content of the water (5.4-10.7 mg/L) and were inversely associated with mean iodine content of the water. (3) Desai et al. (1993) found a positive correlation ($P < 0.001$) between prevalence of goiter (9.5-37.5%) and enamel fluorosis (6.0-59.0%), but no correlation between prevalence of goiter and water iodine concentration ($P > 0.05$)."

The NRC did not have access to a series of Chinese studies that FAN[151] has subsequently translated that provide data on the relationship between fluoride and goitre in communities with either iodine excess, or iodine deficiency. In these studies, fluoride's capacity to increase the goitre rate has been consistently demonstrated,

[151] FAN, Fluoride Action Network. www.fluoridealert.org

suggesting that the relationship between fluoride and goitre is stronger and more easily detected in populations (and individuals) with sub-optimal iodine intakes.

Meng (2013)“ Fluoride, a goitrogenic substance in drinking water, is another contributing factor to high GP. The fluoride concentration of drinking water was as high as 1.00 mg/kg in Chongqing municipality, which led Chongqing to have the highest GP (18.37%, 18 of 98) amongst all study areas.”[152]

Gas’kov (2005)“ Analysis of the simultaneous action of factors of the environment (iodine deficits and fluorosis) has shown that the basic cause of enlargement of the thyroid in children is an excessive intake of fluorine. Increasing the amount of iodine absorbed under conditions of excessive intake of fluorine cannot be an effective prophylactic measure directed at the elimination of iodine deficiency states.”[153]

Hong F (2001) “In endemic areas with high fluoride and high iodine, there was greater prevalence of both fluorosis and goiter than in the areas with only one of these two factors. . . . The high fluoride/low iodine group had an increased rate of goiter as compared to low fluoride/low iodine group, possibly stemming from the toxic effects of fluoride interacting with and aggravating the damage caused by a low iodine environment.”[154]

Wang X (2001) “In high iodine and high fluorine areas, the goiter and dental fluorosis rates of children aged from 8 to 12 were clearly higher than the control point, indicating that high iodine and high fluorosis have worse effects on children’s thyroid and teeth.”[155]

Yang (1994) “For children 15 or younger, the rate of thyroid swelling was 29.8% (96/322), and the rate of dental fluorosis reached 72.98% (235/322). In the control group, the rates were 16.13% (15/93) and 18.28 (17/93), respectively, with P<0.01 in all cases, indicating that the harm caused by a high fluoride-high iodine environment

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[152] Meng F, et al. (2013). Assessment of iodine status in children, adults, pregnant women and lactating women in iodine-replete areas of China. PLoS One 8(11):e81294.
[153] Gas’kov A, et al. (2005). The specific features of the development of iodine deficiencies in children living under environmental pollution with fluorine compounds. *Gig Sanit.* Nov-Dec;(6):53-5.
[154] Hong F, et al. (2001). Research on the effects of fluoride on child intellectual development under different environments. Chinese Primary Health Care 15(3):56-57 (republished in Fluoride 2008; 41(2):156–60).
[155] Wang X, et al. (2001). Effects of high iodine and high fluorine on children’s intelligence and thyroid function. Chinese Journal of Endemiology 20(4):288-90.

is particularly serious in the case of children."[156]

Lin F (1986) "In the lower alluvial plains, endemic goiter occurred concomitantly with endemic fluorosis and the contents of iodine in both water and urine were higher, but did not reach the level found in countries where goiter could be attributed to excess intake of iodine.The fact that in the circumstances of the lower uptake of I in thyroid for 24 hours and normal values of T3, T4, TSH, endemic goiter still was slightly prevalent indicated that fluoride also was a factor responsible for goiter."[157]

Jooste (1999) "OBJECTIVE: The study was undertaken to investigate whether endemic goitre still exists in the Northern Cape Province of South Africa more than 55 years after it was reported and, if so, whether iodine deficiency, or fluoride in the drinking water, is linked to the goitres. DESIGN: Cross-sectional study of children in three pairs of towns. SUBJECTS: The 6-, 12- and 15-year-old children (n = 671) who had been lifetime residents in two Northern Cape towns with low levels, two towns with near optimal levels and two towns with high levels of fluoride in the drinking water were recruited through the schools as study participants. RESULTS: Endemic goitre was found in all the towns except one, ranging from 5% to 29%. Iodine deficiency did not prevail in the study area because the median urinary iodine concentration, exceeding 1.58 micromol/l in all but one of the towns, indicated a more than adequate iodine consumption. The drinking water and, to a lesser extent, iodised salt were important sources of iodine. No relationship was found between fluoride in the water and the mild goitre prevalence (5% to 18%) in the four towns with either a low or near optimal fluoride content in the water. The causal factor(s) responsible for the goitres in these four towns were not clear from our data. However, the prevalence of goitre was higher (28% and 29%) in the two towns with high levels of fluoride in the water. CONCLUSION: These results indicate that either a high fluoride level in the water or another associated goitrogen, other than iodine deficiency, may have been responsible for these goitres."[158]

Desai (1993) "We examined 22,276 individuals for presence of goitre and dental fluorosis and estimated the fluoride and iodine content of their drinking water. Overall goitre and dental fluorosis prevalences were 14.0% and 12.2%, respectively, and were significantly and positively correlated. No significant relationship was observed between water iodine level and goitre. In the study area only 0.3% of cases were visible goitre (Grade-II and above) and all goitre cases were euthyroid.

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[156] Yang Y, et al. (1994). The effects of high levels of fluoride and iodine on intellectual ability and the metabolism of fluoride and iodine. Chinese Journal of Epidemiology 15(4):296-98 (republished in Fluoride 2008; 41:336-339).

[157] Lin F, et al. (1986). A preliminary approach to the relationship of both endemic goiter and fluorosis in the valley of Manasi River, Xin-Jiang to environmental geochemistry. Chinese Journal of Endemiology 5(1):53-55.

[158] Jooste PL, et al. (1999). Endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province of South Africa. European Journal of Clinical Nutrition 53(1):8-12.

This suggests that fluoride-induced goitres are brought about by anatomical or structural changes rather than functional changes."[159]

Obel (1982)" Areas which have endemic goitre in Kenya are highlands in the central parts of the country where there are no lakes from which iodide-rich foodstuffs, such as fish, could be found. Iodized salt has been mandatorily available in Kenya for many years. Indeed, most of the cases of goitre from these areas do not show iodide deficiency on biochemical evaluation. Many of these patients manifest clinical and laboratory findings of simple goitre (normal plasma levels of thyroxine, triiodothyronin, thyroid stimulating hormone, and normal iodine uptake values). It therefore would appear unlikely that absolute iodide deficiency per se would account for endemic goitre in Kenya. . . . It is interesting that the same areas which suffer from endemic goitre in Kenya also have the highest prevalence of fluorosis in the country. Indeed, many cases of fluorosis in Kenya have concurrent fluorosis."[160]

Day (1972). "The prevalence of goitre in 17 Himalayan villages has been estimated. Water-samples from each village were taken, and levels of iodine, fluoride, and hardness determined. In 13 villages wide variations in goitre prevalence were not attributable to differences in iodine intake, which remained constant within a narrow range. Instead, variations in goitre prevalence were found to correlate closely with the fluoride content (p=0-74; P<0-01) and with the hardness (p=0.77; P<0-01) of the water in each village. The effects of fluoride and water hardness seem to be independent."[161]

Siddiqui (1969) "With regard to the slight and temporary enlargement of the thyroid encountered in the age group 14-17 (type b), detailed scrutiny of the data . . . reveals that with a fall in mean fluorine content of the water from 10.7 mg/l in Kamaguda to 5.4 mg/l in Yellareddyguda, there was a corresponding progressive fall in the incidence of pubertal goiters from 40% in Kamaguda to 9% in Yellareddyguda, However, associated with the fall in fluorine content there was also a rise in mean iodine of the water. The figures can be interpreted to indicate that, so far as type b goiters are concerned, (1) fluorine may be actually goitrogenic, and (2) high concentrations of iodine may have a goiter-preventing effect. Investigations in other areas, where the variations in fluorine content are not associated with variations in iodine content of the type encountered here, may throw light on this particular problem."[162]

Steyn DG, et al. 1955. In 1936 while on an investigation into poisoning of man and animal by subterranean waters in the North-Western Cape Province, one of us

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[159] Desai VK, et al. (1993). Epidemiological study of goitre in endemic fluorosis district of Gujarat. Fluoride. 26(3):187-90.
[160] Obel AO. (1982). Goitre and fluorosis in Kenya. East African Medical Journal 59:363-365.
[161] Day TK, Powell-Jackson PR. (1972). Fluoride, water hardness, and endemic goitre. Lancet 1:1135-1138.
[162] Siddiqui AH. (1969). Incidence of simple goiter in areas of endemic fluorosis in Nalgonda district, Andhra Pradesh, India. Fluoride 2(2): 200-05.

[D.G.S. (126-129)] encountered several cases of goitre in European women living on farms. Enquiries made, revealed that a fair percentage of people, especially women, settling in this part of the country developed enlargement of the thyroid gland within 10 to 15 years after having entered the area. This was a puzzling phenomenon as the North Western Cape Province is known to be rich in iodine. It was realized that endemic goitre in this area could not possibly be the result of primary iodine deficiency in the soil, food and water. It was thought that the cause must be sought in the drinking water. The area is semi-arid and all drinking water, except that of towns and farms situated on the Orange River, is drawn from wells and boreholes. It was also known that the subterranean waters in the North-Western Cape Province generally contain harmful quantities of fluorine. It was considered that there was a possibility that fluorine has an antithyroid (goitrogenic) action. After having consulted the literature and conducting some experiments upon rats, it was realized that fluorine is a goitrogenic agent and that endemic goitre in the North-Western Cape Province is due not to an inherent primary iodine deficiency but chiefly to the general presence of harmful quantities of fluorine in the drinking-water. It is possible that the large quantities of calcium generally present in the subterranean waters in that area, enhances the goitrogenic effect of fluorine. Generally speaking the diet of the people is very satisfactory as it included a good percentage of meat with vegetables, fruit and bread. A large percentage of the vegetables and fruit is imported."[163]

Wilson (1941) "The distribution of endemic goitre in the Punjab and in England is related to the geological distribution of fluorine and to the distribution of human dental fluorosis (mottled enamel). Inquiry showed the presence of dental fluorosis among school-children in two areas of Somerset where two previous observers had recorded a high incidence of goitre, and the absence of dental fluorosis in an adjoining area selected as control where endemic goitre was absent."[164]

[163] Steyn DG, et al. 1955. Endemic goitre in the Union of South Africa and some neighbouring territories. Union of South Africa. Department of Nutrition.

[164] Wilson DC. (1941). Fluorine in the aetiology of endemic goitre. The Lancet 15(6129): 212-213.

Liu H (2013) “Excessive iodide and fluoride coexist in the groundwater in many regions, causing a potential risk to the human thyroid. To investigate the mechanism of iodide- and fluoride-induced thyroid cytotoxicity, human thyroid follicular epithelial cells (Nthy-ori 3-1) were treated with different concentrations of potassium iodide (KI), with or without sodium fluoride (NaF). . . . Collectively, excessive iodide and/or fluoride is cytotoxic to the human thyroid. Although these data do not manifest iodide could induce the IRE1 pathway, the cytotoxicity followed by exposure to fluoride alone or in combination with iodide may be related to IRE1 pathway-induced apoptosis. Furthermore, exposure to the combination of excessive iodide and fluoride may cause interactive effects on thyroid cytotoxicity.”[165]

Liu (2012) “Endemic fluorosis is a serious problem in public health. Previous studies have indicated that patients with thyroid goiters usually live in fluoride-affected areas. However, the mechanism of goitrogenesis caused independently by fluoride is still unclear. The principle objective of this study was to investigate the possible roles of nitric oxide (NO) and vascular endothelial growth factor (VEGF) in the genesis of fluoride-induced nodular goiters. . . . The results showed that the average relative weight of the thyroid glands of rats in the fluoride-treated groups was significantly higher than that in control rats ($p<0.05$). The proliferation and dilatation of capillary blood vessels, enlarged follicles with excessive colloid, and obvious nodules were found in the thyroid glands of fluoride-treated rats. Compared to the control group, the expression of VEGF mRNA in the thyroid gland and the serum NO levels in the fluoride-treated groups were significantly increased ($p<0.05$). Furthermore, the deposition of VEGF in epithelial and follicular cells of the thyroid gland was significantly higher in fluoride-treated groups than in the control group. These results suggested that abnormal expression of VEGF induced by fluoride can lead to the proliferation of vascular endothelial cells in the thyroid gland. Accordingly, VEGF oversecreted locally by vascular endothelial cells might contribute to the proliferation of epithelial and follicular cells, resulting in the formation of hyperplastic nodules and enlargement of the thyroid gland. Furthermore, we proposed that there might be a positive feedback mechanism between NO and VEGF expression in fluoride-induced goiter formation. It was concluded that angiogenic and vasodilative factors such as VEGF and NO must be involved in fluoride-induced thyroid goitrogenesis.”[166]

Zeng Q (2012) “To explore the toxic effect of fluoride on the human thyroid cells (Nthy-ori 3-1) and its mechanism. . . . To Nthy-ori 3-1 cells, fluoride under experimental concentrations decreases cell viability, improve the LDH leakage rate, and ROS level. It

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[165]Liu H et al, The role of the IRE1 pathway in excessive iodide- and/or fluoride-induced apoptosis in Nthy-ori 3-1 cells in vitro. Toxicol Lett. 2014 Jan 30;224(3):341-8. doi: 10.1016/j.toxlet.2013.11.001. Epub 2013 Nov 11.

[166] Liu G[1], Zhang W, Jiang P, Li X, Liu C, Chai C. Role of nitric oxide and vascular endothelial growth factor in fluoride-induced goitrogenesis in rats. Environ Toxicol Pharmacol. 2012 Sep;34(2):209-17. doi: 10.1016/j.etap.2012.04.003. Epub 2012 Apr 10.

blocks the cells in S phase and induce cell apoptosis."[167]

Liu (2012) "Endemic fluorosis is a serious problem in public health. Previous studies have indicated that patients with thyroid goiters usually live in fluoride-affected areas. . . It was concluded that angiogenic and vasodilative factors such as VEGF and NO must be involved in fluoride-induced thyroid goitrogenesis."[168]

Bashar (2011) "High-fluoride (100 and 200 ppm) water was administered to rats orally to study the fluoride-induced changes on the thyroid hormone status, the histopathology of discrete brain regions, the acetylcholine esterase activity, and the learning and memory abilities in multigeneration rats. Significant decrease in the serum-free thyroxine (FT4) and free triiodothyronine (FT3) levels and decrease in acetylcholine esterase activity in fluoride-treated group were observed. Presence of eosinophilic Purkinje cells, degenerating neurons, decreased granular cells, and vacuolations were noted in discrete brain regions of the fluoride-treated group. In the T-maze experiments, the fluoride-treated group showed poor acquisition and retention and higher latency when compared with the control. The alterations were more profound in the third generation when compared with the first- and second-generation fluoride-treated group. Changes in the thyroid hormone levels in the present study might have imbalanced the oxidant/antioxidant system, which further led to a reduction in learning memory ability. Hence, presence of generational or cumulative effects of fluoride on the development of the offspring when it is ingested continuously through multiple generations is evident from the present study."[169]

Cai (2009) "Objective: To observe the effects of fluoride on thyroid morphology, thyroid peroxidase and serum thyroid hormones. Methods: One-month ablactating SD rats were randomly divided into groups: the control group low-fluoride group, middle-fluoride group, high-fluoride group; fed with water containing different fluoride concentration by adding NaF respectively. Rats were sacrificed after being fed for six months. The morphology of thyroid was observed through light microscope. The TPO activity was measured with upgrade guaiacol method. Radio-immunoassay

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[167] Zeng Q et al. [Studies of fluoride on the thyroid cell apoptosis and mechanism]. [Article in Chinese] Journal; Zhonghua Yu Fang Yi Xue Za Zhi. 2012 Mar;46(3):233-6.

[168] Liu G, Zhang W, Jiang P, Li X, Liu C, Chai C. Role of nitric oxide and vascular endothelial growth factor in fluoride-induced goitrogenesis in rats. Environ Toxicol Pharmacol. 2012 Sep;34(2):209-17. doi: 10.1016/j.etap.2012.04.003. Epub 2012 Apr 10.

[169] Basha PM[1], Rai P, Begum S. Fluoride toxicity and status of serum thyroid hormones, brain histopathology, and learning memory in rats: a multigenerational assessment. Biol Trace Elem Res. 2011 Dec;144(1-3):1083-94. doi: 10.1007/s12011-011-9137-3. Epub 2011 Jul 14.

was used to detect serum thyroid hormones. Results: The major changes included increased follicles with colloid accumulation in high fluoride groups. With the dose of fluoride increasing, TPO activity significantly decreased as compared with the control group (P0.05). FT4 levels of the high-fluoride were significantly lower compared with the control group (P0.05). Conclusions: Chronic fluoride excess leads to definite histological changes in rat thyroid, inhibiting TPO activity so that level of thyroid hormone is decreased,which shows that fluoride can cause goiter, and cause abnormal changes of thyroid metabolism function."[170]

Zang (2008) "To investigate the mechanism of goiter caused by fluoride, goiter model of SD rats was produced by administering sodium fluoride in drinking water. Histological section of thyroid gland was made, and inducible nitric oxide synthase (iNOS) and vessel endothelial growth factor (VEGF) were determined by RT-PCR. Results showed that the capillary vessels in thyroid glands of the rats treated with fluoride proliferated and an obvious nodular goiter occurred in the fluoride-treated rats. Compared with the control, the contents of iNOS and VEGF in the thyroid glands of the rats with fluorosis was increased significantly (P0.05).It was concluded from the results that the mechanism of goiter caused by fluoride was that fluoride induced the over-expressions of iNOS and VEGF mRNAs in thyroid gland, which caused hyperplasia of capillary vessels."[171]

Shen (2004) "OBJECTIVE: Investigating the influence of combined iodine and fluoride on phospholipid and fatty acid composition in brain cells of rats. METHODS: Five groups of rats were provided with deionized drinking water containing 0 and 150 mg/L NaF, and containing both 150 mg/L NaF and 0.003, 0.03 or 3 mg/L KI respectively for 5 months. Then phospholipid and fatty acid composition were determined using liquid chromatography. RESULTS: The phospholipid composition had no obvious change. The high concentration fluoride (150 mg/L) and high concentration Iodine (3 mg/L) with high concentration fluoride could cause significant changes of the fatty acid composition in brain cells of rats, the proportion of unsaturated fatty acid (C18:2) was significantly decreased and the saturated fatty acid (C12:0) increased obviously. The antagonistic action of 0.03 mg/L KI drinking water on this kind of influence induced by 150 mg/L NaF was the most evident, whereas that of 3 mg/L KI was action of synergetic toxicity. CONCLUSION: Fluorosis had obvious influence on phospholipid and fatty acid composition in brain cells of rats, and its mechanism might be associated with action of lipid peroxidation, and 0.03 mg/L KI is the optimal concentration for the antagonistic action with this influence from fluorosis."[172]

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[170] Cai Q, Li Hong. (2009). Effects of Fluoride on the Thyroid Morphology and Thyroid Peroxidase and Serum Thyroid Hormones. Journal of Liaoning Medical University.

[171] Zhang W, et al. (2008). Expressions of iNOS and VEGF mRNAs in thyroid gland of rat with goiter induced by fluoride. Chinese Veterinary Science.

[172] Shen X, et al. (2004). [Influence of combined iodine and fluoride on phospholipid and fatty acid composition in brain cells of rats]. Wei Sheng Yan Jiu 33(2):158-61. [Article in Chinese]

Zhao (1998) “fluorine also affected the thyroid changes induced by ID [iodine deficiency] or IE [iodine excess]. After 100 days of treatment, fluorine showed some stimulatory effect on the thyroid in ID conditions and inhibitory effect in IE conditions. After 150 days, however, the effects of fluorine on the thyroid reversed as compared with that of 100 days. On the other hand, difference of iodide intake could also increase the toxic effects of FE on the incisors and bones.”[173]

Burg (1984)[174] Burgi and colleagues published a critique of then-existing research linking fluoride to thyroid dysfunction, including goitre and included studies which failed to find a relationship between fluoride and goiters.

[173] Zhao W, et al. (1998). Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. Endocrine Regulation 32(2):63-70.
Environ Toxicol Pharmacol. 2014 Jul;38(1):332-40. doi: 10.1016/j.etap.2014.06.008. Epub 2014 Jun 27.

[174] Burgi H, et al. (1984): Fluorine and the Thyroid Gland: A Review of the Literature. *Klin Wochenschr*. 1984 Jun 15;62(12):564-9.

Bill Osmunson DDS, MPH
Audrey Adams

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
GENERAL COUNSEL

February 14, 2013

Gerald Steel, PE
7303 Young Road NW
Olympia, WA 98502

Dear Mr. Steel:

This is in response to your letter of December 28, 2012 to EPA Administrator Lisa Jackson in which you asked several questions about the status of an MOU between EPA and the Federal Drug Administration (FDA) published in 1979. I am replying on behalf of her.

Your first question is whether, from the viewpoint of EPA, the purpose of a 1979 Memorandum of Understanding (MOU) between EPA and the Federal Drug Administration (FDA) was "to take away from FDA, and give to EPA, responsibility for regulating public drinking water additives intended for preventative health care purposes and unrelated to contamination of public drinking water?" Your second question is whether, if that was the purpose of the 1979 MOU, the MOU was terminated through a subsequent Federal Register notice.

The answer to your first question is no, so there is no need to address your second question. The purpose of the MOU was not to shift any responsibilities between the Agencies. Rather, it was to help facilitate effective coordination of our respective legal authorities. Under the Safe Drinking Water Act (SDWA), EPA is the lead federal agency with responsibility to regulate the safety of public water supplies. EPA does not have responsibility for substances added to water solely for preventative health care purposes, such as fluoride, other than to limit the addition of such substances to protect public health or to prevent such substances from interfering with the effectiveness of any required treatment techniques. SDWA Section 1412(b)(11); see also A Legislative History of the Safe Drinking Water Act, Committee Print, 97th Cong, 2d Session (February 1982) at 547. The Department of Health and Human Services (HHS), acting through the FDA, remains responsible for regulating the addition of drugs to water supplies for health care purposes.

The 1979 MOU was intended to address contamination of drinking water supplies as a result of direct or indirect additives to drinking water, not to address the addition of substances solely for preventative health purposes. 44 Fed. Reg. 42775 (July 20, 1979) ("EPA and FDA agree: (1) that *contamination* of drinking water from the use and application of direct and indirect additives and other substances poses a potential public health problem...")(emphasis added). It was intended to avoid potentially duplicative regulation of "food", which FDA had, in the past, considered to include drinking water. 44 Fed. Reg. 42775 (July 20, 1979). The MOU did not address drugs or other substances added to water for health care purposes.

I hope that this has adequately answered your inquiry. Please do not hesitate to contact Carrie Wehling of my staff (202-564-5492) if you have further questions about this.

Sincerely,

Steven M. Neugeboren
Associate General Counsel
Water Law Office

WASHINGTON STATE
Board of Health
ALWAYS WORKING FOR A SAFER AND HEALTHIER WASHINGTON

DATE: June 9, 2010

TO: Washington State Board of Health Members

FROM: Environmental Health Committee:
Karen VanDusen, Keith Higman, and John Austin

SUBJECT: **PETITION FOR RULE MAKING: WATER FLUORIDATION, WAC 246-290-220 AND WAC 246-290-460**

Background and Summary:

On May 11, 2010, the Washington State Board of Health received a petition for rule making in the form of an e-mailed letter from Bill Osmunson, DDS, MPH, president of Washington Action for Safe Water. The petition asks the Board to amend WAC 246-290-460 and WAC 246-290-220, sections in the Board's rules for Group A public water supplies. The first requested amendment would change the allowable concentration of a fluoridation additive from a range specified in rule to a range approved by the U.S. Food and Drug Administration (FDA). The second would change the requirement that drinking water fluoridation additives meet Standard 60 of the National Sanitation Foundation (NSF) and American National Standards Institute (ANSI) to a requirement the additives be approved by FDA under a New Drug Application.

RCW 34.05.330 provides the opportunity for anyone to petition the Board with a request to adopt, amend, or repeal any of its rules. Upon receipt of such a petition, the Board has sixty days to initiate rule making, deny the petition, or address concerns raised by the petitioner by alternate means. Board policy number 2005-001 sets forth the procedures followed by the Board when it receives such a request. According to this policy, the chair may either decide on the request and instruct the executive director to respond or take the request to the full Board for discussion and possible action.

Chair Higman has worked with the Board's Environmental Health (EH) Committee to review the petition and make a recommendation for action. Ned Therien, Board staff, will summarize this rule making petition and EH Committee recommendations for the Board. Please refer to materials behind Tab 16 for additional information.

Recommended Board Action

The Environmental Health Committee recommends the Board adopt the following motion:

> ***Motion**: The Board denies the petition for rule making from Dr. William Osmunson dated May 11, 2010 because the U.S. Food and Drug Administration has a memorandum of understanding with the U.S. Environmental Protection Agency clarifying that the latter agency has authority for regulating tap water.*

Discussion:

The Board has authority under RCW 43.20-050(2) to adopt rules for Group A public water supplies "necessary to assure safe and reliable public drinking water and to protect public health." The Board has further responsibility under RCW 70.142.010 to establish standards for chemical contaminants in public drinking water and "consider the best available scientific information in establishing the standards." The Board has adopted such rules in chapter 246-290 WAC. These rules set both a maximum contaminant level (MCL) for fluoride in drinking water and a lower allowable concentration range if fluoride is added to drinking water. These rules also require that drinking water additives meet NSF/ANSI Standard 60.

RCW 57.08.012 gives each water district the authority to decide whether to ask the electors of the water district to vote on adding fluoride to its tap water. The Board does not appear to have authority to adopt rules related to a water district deciding whether to fluoridate. The Board's authority is to regulate allowable concentration levels and method of approval of water additives.

Dr. Osmunson asked the Board of Pharmacy in 2009 to designate fluoride a poison under chapter RCW 69.38 RCW, Poisons—sales and manufacturing. Dr. Osmunson asserted that fluoridation of public water supplies was the therapeutic administration of fluoride and should be controlled by the laws for legend drugs. The Pharmacy Board's response was that RCW 57.08.012, by being more specific, supersedes the general statutory authority under which it regulates drugs.

For fluoride in drinking water, this Board has adopted the U.S. Environmental Protection Agency (EPA) primary MCL of 4 parts per million (ppm) and secondary MCL of 2 ppm under WAC 246-290-310. These standards are primarily intended for naturally occurring fluoride. The Board has adopted under WAC 246-290-460 an allowable concentration range for artificial fluoridation of public tap water. This range is 0.8–1.3 ppm and is based on the Centers for Disease Control and Prevention (CDC) "optimal" recommended levels to help prevent tooth decay. The Board has adopted under WAC 246-290-220 requirements that drinking water additives meet NSF/ANSI Standard 60. These organizations have developed these standards in association with EPA and the American Water Works Association.

CDC recommends public tap water be fluoridated to an "optimal" target concentration of 0.7–1.2 ppm to help prevent cavities. This is a range of target concentrations and the actual target for a given water supplier would be based on a five-year average of the maximum daily air temperature for the supplier's service area. CDC recommends the concentration be controlled within a range no less than 0.1 ppm below and no more than 0.5 ppm above a supplier's target concentration. For example, if the target concentration is determined to be 0.9 ppm, the control range would be between 0.8 ppm and 1.4 ppm. The Board's standard of 0.8–1.3 ppm in WAC 246-290-460 was set based on different target concentrations across the state, which fall between 0.9 ppm and 1.1 ppm. The allowable range permits a variation of no more than 0.4 above the target concentration for the warmest part of the state. Therefore, the Board's rule is more stringent than the CDC recommendation.

The National Research Council (NRC) Committee on Fluoride in Drinking Water issued a report in 2006 titled *FLUORIDE IN DRINKING WATER: A Scientific Review of EPA's Standards.* It

recommended the MCL for fluoride be lowered from 4 ppm, but did not recommend a new level. It concluded that 2 ppm seemed safe, but might be high enough to cause moderate tooth discoloration (less that 15% of children). It did not specifically address the issue of the CDC-recommended 0.7 - 1.2 ppm concentration range for adding fluoride to a water supply. On March 29, 2010, EPA published in the *Federal Register* an announcement of a six-year review of the MCLs for 71 chemicals, one of which was fluoride. It requested public comments on the reviews by May 28, 2010. EPA's conclusion is that it does not have information at this time that warrants it making a change to the MCL for fluoride, but studies are continuing.

CDC considers drinking water fluoridation one of the top ten great public health achievements of the 20th century. A series of surgeon general statements, the last issued in 2004, have strongly supported fluoridation of community water systems. CDC states that the 2006 National Research Council report supports CDC's recommended "optimal" fluoridation levels as being safe. CDC further states that the most common chemical used for fluoridation, fluorosilicic acid, and related compounds are derived in high purity from the gypsum and phosphate fertilizer manufacturing process. CDC cautions against the overuse of fluoride-containing products to control total intake. In a telephone call between Ned Therien and William Bailey, DDS, MPH, U.S. Public Health Service, on May 21 of this year, Captain Bailey stated that CDC is continually reviewing data regarding the "optimal" level and safety of tap water fluoridation. He also stated that EPA is currently doing risk assessment reviews of dose-response, source contribution, and the potential for carcinogenicity of fluoride.

In 1979, EPA and FDA finalized a memorandum of understanding regarding regulating fluoride levels in drinking water. They concluded the 1974 Safe Drinking Water Act gives EPA authority for regulating chemicals in tap water, while FDA has authority for chemicals in bottled water. Under CFR Title 21, Section 165.110, FDA has set a limit for fluoride added to bottled water in the U.S. of between 0.7 and 1.7 ppm, depending on annual average maximum air temperature for the location where bottled. In a May 21 e-mail exchange between Ned Therien and John V. Kelsey, DDS, MBA, Dental Team Leader, Division of Dermatology and Dental Products, FDA, Dr. Kelsey confirmed that FDA does not have regulatory responsibility for public water supplies, but rather that is the responsibility of EPA. He said if the Board accepted the language proposed in the petition, it effectively would ban public water fluoridation in Washington.

The Washington State Department of Health encourages community water fluoridation as a public health measure. State Health Officer Maxine Hayes, MD, MPH, issued a statement in support of community water fluoridation in 2006. The department's Oral Health Program echoes the recommendations of CDC on community water fluoridation and provides warnings about the overuse of fluoridated products. Many health professional associations support CDC's recommendations on community water fluoridation, including the American Dental Association, American Medical Association, American Academy of Family Physicians, and American Public Health Association.

The EH Committee concludes:

- EPA is the lead federal agency for regulating the maximum levels of contaminants and additives in tap water under the Safe Drinking Water Act.
- FDA has relinquished any authority it might have for regulating fluoride levels in tap water under the memorandum of understanding with EPA.
- The Board cannot direct a federal agency to take action.
- The State Board of Pharmacy has stated it cannot regulate tap water fluoridation under its authority.
- An NRC committee evaluated the scientific evidence of the health effects of fluoride in drinking water and published a report in 2006 that concluded fluoride levels in drinking water below 2 ppm are safe for health.
- EPA announced completion of a review of MCLs in the Federal Register in March 2010 that concluded it did not have evidence to revise the MCL for fluoride.
- EPA will be conducting additional reviews regarding fluoride levels in drinking water.
- EPA recognizes NSF/ANSI Standard 60 as appropriate for the approval of drinking water additives.
- The range of 0.8 ppm to 1.3 ppm fluoride in WAC 246-290-460 is within the control range (0.1 ppm below to 0.5 ppm above) recommended by CDC for target "optimal" concentrations based on average maximum temperatures in various regions of Washington.

The EH Committee recommends the Board deny Dr. Osmunson's petition for rule making on the grounds that FDA has stated it has no intention to regulate fluoride levels or approve additives for tap water. Therefore, adopting the proposed rule changes would, essentially, prohibit all tap water fluoridation in Washington and make Board rules conflict with RCW 57.08.012.

The EH Committee considers much of the discussion in the petition to make points that go beyond the requested rule changes and are not pertinent to its decision. However, the Committee recommends the Department of Health monitor EPA evaluations of safe drinking water levels for fluoride and recommendations from CDC for "optimal" fluoride levels, and that the Department propose rule amendments based on any changes. The Committee further recommends the next time the Department undertakes a major review of chapter 246-290 WAC, it consider proposing the word "optimal" in section 460(3) be changed to a phrase such as "generally regarded as safe." The Committee further recommends the Board continue to review legal points raised in the petition concerning state law and Attorney General opinions.

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Prenatal Fluoride Exposure and Cognitive Outcomes in Children at 4 and 6–12 Years of Age in Mexico

Morteza Bashash,[1] Deena Thomas,[2] Howard Hu,[1] E. Angeles Martinez-Mier,[3] Brisa N. Sanchez,[2] Niladri Basu,[4] Karen E. Peterson,[2,5,6] Adrienne S. Ettinger,[2] Robert Wright,[7] Zhenzhen Zhang,[2] Yun Liu,[2] Lourdes Schnaas,[8] Adriana Mercado-García,[9] Martha María Téllez-Rojo,[9] and Mauricio Hernández-Avila[9]

[1]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada
[2]University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[3]Indiana University School of Dentistry, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana, USA
[4]Faculty of Agricultural and Environmental Sciences, McGill University, Montreal, Quebec, Canada
[5]Center for Human Growth and Development, University of Michigan, Ann Arbor, Michigan, USA
[6]Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[7]Icahn School of Medicine at Mount Sinai, New York, New York, USA
[8]Instituto Nacional de Perinatología, Mexico City, Mexico
[9]Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico

Background: Some evidence suggests that fluoride may be neurotoxic to children. Few of the epidemiologic studies have been longitudinal, had individual measures of fluoride exposure, addressed the impact of prenatal exposures or involved more than 100 participants.

Objective: Our aim was to estimate the association of prenatal exposure to fluoride with offspring neurocognitive development.

Methods: We studied participants from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project. An ion-selective electrode technique was used to measure fluoride in archived urine samples taken from mothers during pregnancy and from their children when 6–12 y old, adjusted for urinary creatinine and specific gravity, respectively. Child intelligence was measured by the General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities at age 4 and full scale intelligence quotient (IQ) from the Wechsler Abbreviated Scale of Intelligence (WASI) at age 6–12.

Results: We had complete data on 299 mother–child pairs, of whom 287 and 211 had data for the GCI and IQ analyses, respectively. Mean (SD) values for urinary fluoride in all of the mothers ($n = 299$) and children with available urine samples ($n = 211$) were 0.90 (0.35) mg/L and 0.82 (0.38) mg/L, respectively. In multivariate models we found that an increase in maternal urine fluoride of 0.5 mg/L (approximately the IQR) predicted 3.15 (95% CI: −5.42, −0.87) and 2.50 (95% CI −4.12, −0.59) lower offspring GCI and IQ scores, respectively.

Conclusions: In this study, higher prenatal fluoride exposure, in the general range of exposures reported for other general population samples of pregnant women and nonpregnant adults, was associated with lower scores on tests of cognitive function in the offspring at age 4 and 6–12 y. https://doi.org/10.1289/EHP655

Introduction

Community water, salt, milk, and dental products have been fluoridated in varying degrees for more than 60 y to prevent dental caries, while fluoride supplementation has been recommended to prevent bone fractures (Jones et al. 2005). In addition, people may be exposed to fluoride through the consumption of naturally contaminated drinking water, dietary sources, dental products, and other sources (Doull et al. 2006). Whereas fluoride is added to drinking water [in the United States at levels of 0.7–1.2 mg/L (Doull et al. 2006)] to promote health, populations with exceptionally high exposures, often from naturally contaminated drinking water, are at risk of adverse health effects, including fluorosis.

In the United States, the U.S. Environmental Protection Agency (EPA) is responsible for establishing maximum permissible concentrations of contaminants, including fluoride, in public drinking-water systems. These standards are guidelines for restricting the amount of fluoride contamination in drinking water, not standards for intentional drinking-water fluoridation. In 2006 the U.S. EPA asked the U.S. National Research Council (NRC) to reevaluate the existing U.S. EPA standards for fluoride contamination, including the maximum contaminant level goal (MCLG, a concentration at which no adverse health effects are expected) of 4 mg/L, to determine if the standards were adequate to protect public health (Doull et al. 2006). The committee concluded that the MCLG of 4 mg/L should be lowered because it puts children at risk of developing severe enamel fluorosis, and may be too high to prevent bone fractures caused by fluorosis (Doull et al. 2006). The Committee also noted some experimental and epidemiologic evidence suggesting that fluoride may be neurotoxic (Doull et al. 2006).

The National Toxicology Program (NTP) recently reviewed animal studies on the effects of fluoride on neurobehavioral outcomes and concluded that there was a moderate level of evidence for adverse effects of exposures during adulthood, a low level of evidence for effects of developmental exposures on learning and memory, and a need for additional research, particularly on the developmental effects of exposures consistent with those resulting from water fluoridation in the United States (Doull et al. 2006; NTP 2016). Human studies have shown a direct relationship between the serum fluoride concentrations of maternal venous blood and cord blood, indicating that the placenta is not a barrier to the passage of fluoride to the fetus (Shen and Taves, 1974). Fluoride was shown to accumulate in rat brain tissues after chronic exposures to high levels, and investigators have speculated that accumulation in the hippocampus might explain effects on learning and memory (Mullenix et al. 1995). An experimental study on mice has shown that fluoride exposure may have adverse effects on neurodevelopment, manifesting as both cognitive and behavioral abnormalities later in life (Liu et al. 2014).

Please send correspondence to M. Bashash, Dalla Lana School of Public Health, 6th floor, 155 College St., Toronto, Ontario M5R3M7 Canada. Telephone: +1-416-978-6512. Email: m.bashash@utoronto.ca

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The authors declare they have no actual or potential competing financial interests.

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Most epidemiologic studies demonstrating associations between fluoride exposure and lower neuropsychological indicators have been conducted in populations living in regions with endemic fluorosis that are exposed to high levels of fluoride in contaminated drinking water. The epidemiologic evidence is limited, however, with most studies using an ecologic design to estimate childhood exposures based on neighborhood measurements of fluoride (e.g., drinking water levels) rather than personal exposure measures. Moreover, almost all existing studies of childhood outcomes are cross-sectional in nature, rendering them weak contributors towards causal inference.

The main objective of this study was to assess the potential impact of prenatal exposures to fluoride on cognitive function and test hypotheses related to impacts on overall cognitive function. We hypothesized that fluoride concentrations in maternal urine samples collected during pregnancy, a proxy measure of prenatal fluoride exposure, would be inversely associated with cognitive performance in the offspring children. Overall, to our knowledge, this is one of the first and largest longitudinal epidemiologic studies to exist that either address the association of early life exposure to fluoride to childhood intelligence or study the association of fluoride and cognition using individual biomarker of fluoride exposure.

Methods

This is a longitudinal birth cohort study of measurements of fluoride in the urine of pregnant mothers and their offspring (as indicators of individual prenatal and postnatal exposures to fluoride, respectively) and their association with measures of offspring cognitive performance at 4 and 6–12 y old. The institutional review boards of the National Institute of Public Health of Mexico, University of Toronto, University of Michigan, Indiana University, and Harvard T.H. Chan School of Public Health and participating clinics approved the study procedures. Participants were informed of study procedures prior to signing an informed consent required for participation in the study.

Participants

Mother–child pairs in this study were participants from the successively enrolled longitudinal birth cohort studies in Mexico City that comprise the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) project. Of the four ELEMENT cohorts [that have been described elsewhere (Afeiche et al. 2011)], Cohort 1 and Cohort 2B recruited participants at birth and did not have archived maternal-pregnancy urine samples required for this analysis; they were thus excluded. Mothers for Cohort 2A ($n = 327$) and 3 ($n = 670$) were all recruited from the same three hospitals in Mexico City that serve low-to-moderate income populations. Cohort 2A was an observational study of prenatal lead exposure and neurodevelopmental outcomes in children (Hu et al. 2006). Women who were planning to become pregnant or were pregnant were recruited during May 1997–July 1999 and were considered eligible if they consented to participate; were ≤ 14 wk of gestation at the time of recruitment; planned to stay in the Mexico City study area for at least 5 y; did not report a history of psychiatric disorders, high-risk pregnancies, gestational diabetes; did not report current use of daily alcohol, illegal drugs, and continuous prescription drugs; and were not diagnosed with preeclampsia, renal disease, circulatory diseases, hypertension, and seizures during the index pregnancy.

Cohort 3 mothers were pregnant women (≤ 14 wk of gestation) recruited from 2001 to 2003 for a randomized trial of the effect of calcium supplementation during pregnancy on maternal blood lead levels (Ettinger et al. 2009). Eligibility criteria were the same as for Cohort 2A, and 670 agreed to participate.

Exposure Assessment

By virtue of living in Mexico, individuals participating in the study have been exposed to fluoridated salt (at 250 ppm) (Secretaría-de-Salud 1995, 1996) and to varying degrees of naturally occurring fluoride in drinking water. Previous reports, based on samples taken from different urban and rural areas, indicate that natural water fluoride levels in Mexico City may range from 0.15 to 1.38 mg/L (Juárez-López et al. 2007; Martínez-Mier et al. 2005). Mean fluoride content for Mexico City's water supply is not available because fluoride is not reported as part of water quality control programs in Mexico.

Mother–child pairs with at least one archived urine sample from pregnancy and measures of neurocognitive function in the offspring were included in this study. In terms of when the archived samples were collected, the pregnant mothers were invited for assessments with the collection of samples during trimester 1 (13.6 ± 2.1 wk for Cohort 3 and 13.7 ± 3.5 wk for Cohort 2A), trimester 2 (25.1 ± 2.3 wk for Cohort 3 and 24.4 ± 2.9 wk for Cohort 2A), and trimester 3 (33.9 ± 2.2 wk for Cohort 3 and 35.0 ± 1.8 wk for Cohort 2A).

A spot (second morning void) urine sample was targeted for collection during each trimester of pregnancy of ELEMENT mothers as well as the offspring children at the time of their measurements of intelligence at 6–12 y old. The samples were collected into fluoride-free containers and immediately frozen at the field site and shipped and stored at -20°C at the Harvard T. H. Chan School of Public Health (HSPH), and then at -80°C at the University of Michigan School of Public Health (UMSPH).

A procedure for urine analysis of fluoride described elsewhere (Martínez-Mier et al. 2011) was adapted and modified for this study. The fluoride content of the urine samples was measured using ion-selective electrode-based assays. First, 3 M sulfuric acid saturated with hexamethyldisiloxane (HMDS) was added to the sample to allow fluoride to diffuse from the urine for 20–24 hr. The diffused fluoride was allowed to collect in 0.05 M of sodium hydroxide on the interior of the petri dish cover. Once the diffusion was complete, 0.25 M of acetic acid was added to the sodium hydroxide to neutralize the solution and then analyzed directly using a fluoride ion-selective electrode (Thermo Scientific Orion, Cat#13-642-265) and pH/ISE meter (Thermo Scientific Orion, Cat#21-15-001). All electrode readings (in millivolts) were calculated from a standard curve. Analyses were performed in a Class 100/1,000 clean room. Quality control measures included daily instrument calibration, procedural blanks, replicate runs, and the use of certified reference materials (Institut National de Santé Publique du Québec, Cat #s 0910 and 1007; NIST3183, Fluoride Anion Standard). Urinary fluoride concentrations were measured at the UMSPH and the Indiana University Oral Health Research Institute (OHRI) as previously described (Thomas et al. 2016). A validation study comparing measures taken by the two labs in the same samples revealed a between-lab correlation of 0.92 (Thomas et al. 2016).

There were a total of 1,484 prenatal samples measured at the UMSPH lab. All of these samples were measured in duplicate. Of these, 305 (20%) of them did not meet the quality control criteria for ion-selective electrode-based methods (i.e., RSD $<20\%$ for samples with F level <0.2 ppm or RSD $<10\%$ when F level >0.2 ppm) (Martinez-Mier et al. 2011). Of these 305, 108 had a second aliquot available and were successfully measured at the OHRI lab in Indiana (sufficient urine volume was not available for the remaining 197 samples). The OHRI lab in Indiana also measured an additional 289 samples. Of the 397

total samples measured at the OHRI lab in Indiana, 139 (35%) were measured in duplicate, for which >95% complied with the quality control criteria above; thus, all 139 values were retained. The remaining 258 (65%) were not measured in duplicate because of limitations in available urine volume, but were included in the study given the excellent quality control at the OHRI lab. In total, we ended up with 1,576 prenatal urine samples with acceptable measures of fluoride.

Of these 1,576 urine samples, 887 also had data on urinary creatinine and were associated with mother–offspring pairs who had data on the covariates of interest and GCI or IQ in the offspring. The urinary creatinine data were used to correct for variations in urine dilution at the time of measurement (Baez et al. 2014). Creatinine-adjusted urinary fluoride concentrations were obtained for each maternally derived sample by dividing the fluoride concentration (MUF) in the sample by the sample's creatinine concentration (MUC), and multiplying by the average creatinine concentration of samples available at each trimester ($MUC_{average}$) using the formula: $(MUF/MUC) \times MUC_{average}$. The values of average creatinine concentration used for the $MUC_{average}$ at each trimester were derived from the larger pool of trimester-1, -2, and -3 samples from Cohorts 2A and 3 examined in our previous report on maternal fluoride biomarker levels (Thomas et al. 2016): 100.81, 81.60, and 72.41 (mg/L), respectively. For each woman, an average of all her available creatinine-adjusted urinary fluoride concentrations during pregnancy (maximum three samples and minimum one sample) was computed and used as the exposure measure (MUF_{cr}). For children, as creatinine measurements were not available, urinary fluoride values (CUF) were corrected for specific gravity (SG) using the formula $CUFsg = CUF(1.02-1)/(SG-1)$ (Usuda et al. 2007).

After calculating MUF_{cr} for the 887 urine samples noted above, 10 values of MUF_{cr} were identified as extreme outliers (>3.5 SDs) and were dropped, leaving 877 measures of MUF_{cr}. These 877 measures of MUF_{cr} stemmed from 512 unique mothers. Of these 512, 71 participants had measurements from each of the three trimesters; 224 had measurements from two of the three trimesters (74, T1 and T2; 131, T1 and T3; and 19, T2 and T3); and 217 had measurements from only one of the trimesters (159, T1; 34, T2; and 24, T3).

Measurement of Outcomes

At age 4 y, neurocognitive outcomes were measured using a standardized version of McCarthy Scales of Children's Abilities (MSCA) translated into Spanish (McCarthy 1991). MSCA evaluates verbal, perceptual-performance, quantitative, memory, and motor abilities of preschool-aged children, and it has previously been successfully used in translated versions (Braun et al. 2012; Julvez et al. 2007; Kordas et al. 2011; Puertas et al. 2010). For this analysis, we focused on the General Cognitive Index (GCI), which is the standardized composite score produced by the MSCA (McCarthy 1991). For children 6–12 y old a Spanish-version of the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler 1999) was administered. WASI includes four subtests (Vocabulary, Similarities, Block Design, and Matrix Reasoning), which provide estimates of Verbal, Performance, and Full-Scale IQ (Wechsler 1999). Both tests were administered by a team of three psychologists who were trained and supervised by an experienced developmental psychologist (L.S.). This team of three psychologists applied all of the McCarthy tests as well as the WASI-FSIQ tests. At the time of follow-up visits (age 4 and 6–12 y), each child was evaluated by one of the psychologists who was blind to the children's fluoride exposure. The inter-examiner reliability of the psychologists was evaluated by having all three psychologists participate in assessments on a set of 30 individuals. For these 30, the inter-examiner reliability of the psychologists was evaluated by calculating the correlation in GCI scores by two of the psychologists with the scores of a third psychologist whom they observed applying the test in all three possible combinations with 10 participants for each observers–examiner pair (i.e., psychologist A (applicant) was observed by psychologist B and psychologist C; psychologist B (applicant) was observed by psychologist A and psychologist C; and psychologist C (applicant) was observed by psychologist A and psychologist B). The mean observer–examiner correlation was 0.99. All raw scores were standardized for age and sex (McCarthy 1991). Inter-examiner reliability was not examined on the WASI test.

Measurement of Covariates

Data were collected from each subject by questionnaire on maternal age (and date of birth), education, and marital status at the first pregnancy visit; on birth order, birth weight, and gestational age at delivery; and on maternal smoking at every prenatal and postnatal visit. Gestational age was estimated by registered nurses. Maternal IQ was estimated using selected subtests of the Wechsler Adult Intelligence Scale (WAIS)-Spanish (Information, Comprehension, Similarities, and Block Design), which was standardized for Mexican adults (Renteria et al. 2008; Wechsler et al. 1981). Maternal IQ was measured at the study visit 6 mo after birth or at the 12-mo visit if the earlier visit was not completed.

The quality of the children's individual home environments was assessed using an age-appropriate version of the HOME score. However, the measure was not available for all observations because it was only added to on-going cohort evaluation protocols beginning in April 2003, when a version of the HOME score instrument that is age-appropriate for children 0–5 y old was adopted, following which a version of the HOME score instrument that is age-appropriate for children ≥6 y old was adopted in September 2009 (Caldwell and Bradley 2003). Thus, we adjusted for HOME score using the measures for 0- to 5-y-old children in the subset of children who had this data in our analyses of GCI, and we adjusted for HOME score using the measures for >6-y-old children in the subset of children who had this data in our analyses of IQ.

Statistical Analyses

Univariate distributions and descriptive statistics were obtained for all exposure variables, outcome variables, and model covariates. For each variable, observations were classified as outliers if they were outside the bounds of the mean ± 3.5 SDs. Primary analyses were conducted with exposure and outcome outliers excluded. Statistical tests of bivariate associations were conducted using chi-square tests for categorical variables and analysis of variance (ANOVA) to compare the means of the outcomes or exposure within groups defined according to the distribution of each covariate. Spearman correlation coefficients were used to measure the correlation between MUF_{cr} and CUF_{sg}. Regression models were used to assess the adjusted associations between prenatal fluoride and each neurocognitive outcome separately. Generalized additive models (GAMs) were used to visualize the adjusted association between fluoride exposure and measures of intelligence [SAS statistical software (version 9.4; SAS Institute Inc.)]. Because the pattern appeared curvilinear, and because GAMs do not yield exact *p*-values for deviations from linearity, we used a Wald *p*-value of a quadratic term of fluoride exposure to test the null hypothesis that a quadratic model fit the data better

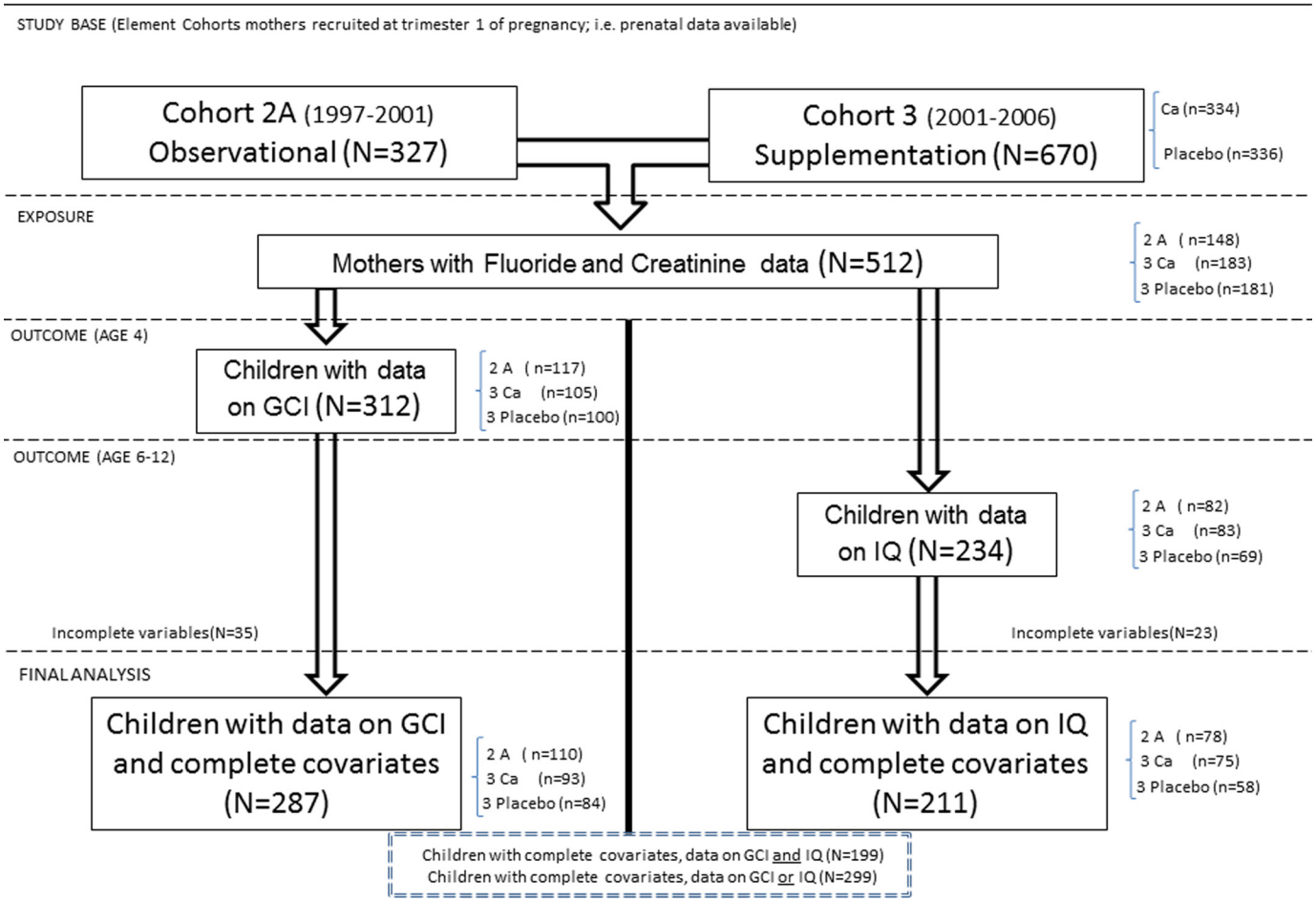

Figure 1. Flowchart describing source of mother–offspring subject pairs, fluoride and cognition study. Cohort 2A was designed as an observational birth cohort of lead toxicodynamics during pregnancy, with mothers recruited early during pregnancy from 1997 to 2001. Cohort 3 was designed as a randomized double-blind placebo-controlled trial of calcium supplements, with mothers recruited early during pregnancy from 2001 to 2006. "Ca" denotes subjects who were randomized to the calcium supplement; "placebo" denotes subjects who were randomized to the placebo. GCI is the McCarthy Scales General Cognitive Index (administered at age 4 y). IQ is the Wechsler Abbreviated Intelligence Scales Intelligence Quotient (administered at age 6–12 y and age-adjusted).

than the model assuming a linear relationship, and thus obtained a *p*-value for deviation from linearity of the fluoride–outcome associations. Residual diagnostics were used to examine other model assumptions and identify any additional potentially influential observations. Visual inspection of default studentized residual versus leverage plot from SAS PROC REG did not identify potential influential observations. Visual inspection of the histogram of the residuals did not indicate lack of normality; however, a fanning pattern in the residual versus predicted value plot indicated lack of constant variance (data not shown). Hence, robust standard errors were obtained using the "empirical" option in SAS PROC GENMOD.

Our overall strategy for selecting covariates for adjustment was to identify those that are well known to have potential associations with either fluoride exposure or cognitive outcomes and/or are typically adjusted for as potential confounders in analyses of environmental toxicants and cognition. All models were adjusted for gestational age at birth (in weeks), birthweight (kilograms), birth order (first born yes vs. no), sex, and child's age at the time of the neurocognitive test (in years). All models were also adjusted for maternal characteristics including marital status (married vs. others), smoking history (ever-smoker vs. never-smoker), age at delivery, IQ, and education (itself also a proxy for socioeconomic status). Finally, all models adjusted for potential cohort effects by including indicator variables denoting from which cohort (Cohort 2A, Cohort 3 + Ca supplement, and Cohort 3 -placebo) the participants came. We used 0.5 mg/L, which was close to the interquartile range of MUF_{cr} for the analyses of both GCI (IQR = 0.45) and IQ (IQR = 0.48), as a standard measure of incremental exposure. SAS statistical software (version 9.4; SAS Institute Inc.) was used for all data analyses described.

Sensitivity Analyses

Models were further adjusted for variables that relate to relatively well-known potential confounders (but for which we were missing a significant amount of data) and variables that were less-well known but possible confounders. The HOME scores were subject to sensitivity analyses because, as noted in the "Methods" section, they were not added to the subject evaluation protocols until 2003, resulting in a significantly smaller subsample of participants with this data. Models of the association between prenatal fluoride exposure (MUF_{cr}) and IQ at 6–12 y old were also adjusted for the child's urine fluoride concentration at 6–12 y of

Table 1. Comparisons across cohorts with respect to the distributions of biomarkers of exposure to prenatal fluoride (MUF_{cr}), prenatal lead (maternal bone Pb), prenatal mercury (maternal blood Hg), and contemporaneous childhood fluoride (CUF_{sg}); and cognitive outcomes (GCI and IQ).

							Percentiles				
Analysis	Measurement	Cohort	*N*	Mean	SD	Min	25	50	75	Max	*p*-Value[a]
GCI Analysis	GCI	Cohort 3-Ca	84	96.88	14.07	50	88	96	107	124	0.997
		Cohort 3-placebo	93	96.80	13.14	50	89	96	105	125	
		Cohort 2A	110	96.95	15.46	56	88	98	110	125	
		Total[b]	287	96.88	14.28	50	88	96	107	125	
	MUF_{cr} (mg/L)	Cohort 3-Ca	84	0.92	0.41	0.28	0.60	0.84	1.14	2.36	0.57
		Cohort 3-placebo	93	0.87	0.34	0.23	0.62	0.82	1.10	2.01	
		Cohort 2A	110	0.92	0.33	0.23	0.68	0.86	1.11	2.14	
		Total[b]	287	0.90	0.36	0.23	0.65	0.84	1.11	2.36	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	62	7.30	7.37	0.05	0.75	4.40	12.93	26.22	<0.01
		Cohort 3-placebo	43	9.21	7.31	0.11	1.50	8.60	13.97	27.37	
		Cohort 2A	62	13.60	11.36	0.15	5.35	10.52	19.46	47.07	
		Total[c]	167	10.13	9.41	0.05	2.37	8.22	15.37	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	38	3.32	1.40	0.73	2.40	3.00	4.15	7.06	0.12
		Cohort 3-placebo	28	2.80	1.33	1.27	1.89	2.53	3.40	7.22	
		Cohort 2A	75	4.53	5.61	0.77	2.30	3.24	4.37	35.91	
		Total[c]	141	3.86	4.25	0.73	2.20	3.08	4.15	35.91	
IQ Analysis	IQ	Cohort 3-Ca	58	94.91	9.86	76	87	96	100	120	0.69
		Cohort 3-placebo	75	96.29	9.63	75	89	97	102	124	
		Cohort 2A	78	96.47	13.20	67	87	96	107	131	
		Total[d]	211	95.98	11.11	67	88	96	107	131	
	MUF_{cr} (mg/L)	Cohort 3-Ca	58	0.89	0.38	0.29	0.57	0.84	1.10	1.85	0.86
		Cohort 3-placebo	75	0.87	0.35	0.23	0.61	0.82	1.11	2.01	
		Cohort 2A	78	0.90	0.34	0.23	0.67	0.85	1.09	2.14	
		Total[d]	211	0.89	0.36	0.23	0.64	0.82	1.07	2.14	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	67	6.97	7.20	0.05	0.76	4.36	11.73	26.22	<0.01
		Cohort 3-placebo	48	9.07	7.42	0.11	1.00	8.49	14.41	27.37	
		Cohort 2A	62	13.60	11.36	0.15	5.35	10.52	19.46	47.07	
		Total[e]	177	9.86	9.33	0.05	2.29	7.95	15.22	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	43	3.25	1.41	0.51	2.43	2.87	4.02	7.06	0.067
		Cohort 3-placebo	31	2.66	1.36	0.78	1.81	2.40	3.26	7.22	
		Cohort 2A	75	4.53	5.61	0.77	2.30	3.24	4.37	35.91	
		Total[e]	149	3.77	4.16	0.51	2.19	2.90	4.11	35.91	
	CUF_{sg} (mg/L)	Cohort 3-Ca	71	0.84	0.4	0.31	0.53	0.78	1.12	2.8	0.29
		Cohort 3-placebo	53	0.85	0.38	0.35	0.57	0.75	1.14	1.85	
		Cohort 2A	65	0.76	0.34	0.18	0.51	0.7	0.89	1.76	
		Total[e]	189	0.82	0.38	0.18	0.54	0.73	1.01	2.8	
All available measurements	GCI	Cohort 3-Ca	133	97.32	13.67	50	88	96	107	124	0.57
		Cohort 3-placebo	149	95.99	13.07	50	88	96	106	125	
		Cohort 2A	150	97.57	14.63	56	88	99	109	131	
		Total[f]	432	96.95	13.80	50	88	96	107	131	
	IQ	Cohort 3-Ca	91	95.92	10.15	76	88	95	103	120	0.92
		Cohort 3-placebo	114	96.56	9.84	75	89	96	102	124	
		Cohort 2A	111	96.25	12.67	67	87	95	105	131	
		Total[f]	316	96.27	10.97	67	88	96	103	131	
	MUF_{cr} (mg/L)	Cohort 3-Ca	181	0.89	0.36	0.28	0.64	0.83	1.09	2.36	0.11
		Cohort 3-placebo	183	0.84	0.31	0.02	0.61	0.81	1.02	2.01	
		Cohort 2A	148	0.91	0.35	0.23	0.67	0.86	1.10	2.15	
		Total[f]	512	0.88	0.34	0.02	0.64	0.82	1.07	2.36	
	Maternal bone Pb (μg/g)	Cohort 3-Ca	97	7.07	7.26	0.01	0.83	4.36	11.78	26.22	<0.01
		Cohort 3-placebo	74	9.15	8.38	0.11	0.85	8.62	13.41	40.8	
		Cohort 2A	86	13.77	11.30	0.15	5.49	10.52	20.58	47.07	
		Total[f]	257	9.91	9.51	0.01	2.01	7.64	15.31	47.07	
	Maternal blood Hg (μg/L)	Cohort 3-Ca	55	3.03	1.41	0.51	2.12	2.77	3.62	7.06	0.09
		Cohort 3-placebo	48	2.87	2.09	0.34	1.82	2.37	3.34	13.47	
		Cohort 2A	104	4.06	4.88	0.77	2.14	3.10	4.16	35.91	
		Total[f]	207	3.51	3.70	0.34	2.07	2.80	3.79	35.91	
	CUF_{sg} (mg/L)	Cohort 3-Ca	104	0.84	0.39	0.31	0.56	0.75	1.07	2.80	0.227
		Cohort 3-placebo	84	0.90	0.46	0.35	0.58	0.75	1.09	2.89	
		Cohort 2A	96	0.79	0.34	0.18	0.53	0.73	0.92	2.11	
		Total[f]	284	0.84	0.40	0.18	0.57	0.74	1.00	2.89	

[a]Analysis of variance across cohorts.
[b]Total number of subjects included in GCI main analysis.
[c]Total number of subjects included in GCI sensitivity analysis.
[d]Total number of subjects included in IQ main analysis.
[e]Total number of subjects included in IQ sensitivity analysis.
[f]Total number of subjects with available measurements, combining Cohort 2A and Cohort 3.

Table 2. Analysis comparing subjects with and without data of interest [n (%) or mean ± SD] with respect to characteristics of mothers and children and sensitivity analysis covariates.

Characteristic	GCI analysis		IQ analysis	
	Included	Excluded	Included	Excluded
Total number[a]	287	710	211	786
Sex				
Female	160 (56%)	244 (47%)	116 (55%)	288 (48%)
Male	127 (44%)	275 (53%)	95 (45%)	307 (52%)
Birth order				
First child	96 (33%)	184 (35%)	93 (32%)	279 (36%)
≥2nd child	191 (67%)	335 (65%)	118 (68%)	507 (65%)
Birth weight (kg)	3.11 ± 0.45	3.11 ± 0.44	3.11 ± 0.46	3.11 ± 0.43
Gestational age (wk)	38.66 ± 1.84	38.58 ± 1.68	38.56 ± 1.80	38.63 ± 1.72
Age at outcome assessment (y)	4.04 ± 0.05	4.05 ± 0.05	8.50 ± 1.31	8.83 ± 1.64
Maternal age at delivery (y)	26.78 ± 5.53	26.49 ± 5.37	27.16 ± 5.61	26.41 ± 5.36
Maternal education (y)[b]	10.63 ± 2.76	10.75 ± 3.08	10.80 ± 2.85	10.69 ± 3.03
Maternal IQ[c]	88.63 ± 12.17	89.27 ± 14.6	89.01 ± 12.45	88.27 ± 13.00
Marital status[d]	3.11 ± 0.45	3.11 ± 0.44	3.11 ± 0.46	3.11 ± 0.43
Married	201 (70%)	493 (70%)	149 (71%)	544 (69%)
Other	86 (30%)	216 (30%)	62 (29%)	240 (31%)
Maternal smoking[e]				
Ever	141 (49%)	335 (51%)	102 (48%)	374 (51%)
Never	146 (51%)	325 (49%)	109 (52%)	362 (49%)
Cohort				
Cohort 3-Ca	93 (32%)	241 (34%)	76 (36%)	259 (33%)
Cohort 3-placebo	84 (29%)	252 (36%)	59 (28%)	278 (35%)
Cohort 2A	110 (38%)	217 (31%)	78 (37%)	249 (32%)
Sensitivity Analyses				
HOME score[f]	$N^{\dagger} = 138$	$N^{\ddagger} = 87$	$N^{\dagger} = 124$	$N^{\ddagger} = 55$
	35.24 ± 6.31	33.23 ± 6.55	35.54 ± 7.46	35.8 ± 7.44
SES[g]	$N^{\dagger} = 188$	$N^{\ddagger} = 110$	$N^{\dagger} = 199$	$N^{\ddagger} = 98$
	6.35 ± 2.43	6.94 ± 2.72	6.36 ± 2.41	6.98 ± 2.79
Maternal Bone Pb (μg/g)[h]	$N^{\dagger} = 167$	$N^{\ddagger} = 91$	$N^{\dagger} = 177$	$N^{\ddagger} = 80$
	9.26 ± 10.55	8.97 ± 10.32	9.02 ± 10.43	9.48 ± 10.55
Maternal Blood Hg (μg/L)[i]	$N^{\dagger} = 141$	$N^{\ddagger} = 67$	$N^{\dagger} = 149$	$N^{\ddagger} = 58$
	3.86 ± 4.25	2.76 ± 1.95	3.77 ± 4.16	2.83 ± 2.01
CUF_{sg}[j] (mg/L)			$N^{\dagger} = 124$	$N^{\ddagger} = 55$
			35.54 ± 7.46	35.8 ± 7.44

[a]The total number of subjects ($n = 997$) are all mother–offspring pairs who participated in the original Cohort 2A and Cohort 3 studies.
[b]Maternal education at the time of the child's birth.
[c]Maternal IQ measured at 6 mo after child's birth.
[d]Mother's marital status at the time of the child's birth.
[e]History of any maternal smoking.
[f]HOME score measured using the separate age-appropriate instruments pertaining to children of ≤5 y old; and children >5 y old.
[g]Family socioeconomic status (SES) measured by questionnaire of family possessions at follow-up.
[h]Maternal patella bone lead measured by KXRF after birth.
[i]Maternal average blood mercury during pregnancy.
[j]Children's specific gravity–corrected urinary fluoride measured at the time of each child's IQ test (6–12 y old).
$N^{\dagger}$ Number of subjects with measurements of MUF_{cr}, cognitive outcome, main covariates, and sensitivity covariates (they are included in the sensitivity model).
$N^{\ddagger}$ Number of subjects with measurements of sensitivity covariates, but missing data on exposure, outcomes, or main covariates (they are excluded from the sensitivity model).

age (CUF_{sg}), a measure that was collected in a significantly smaller subset of individuals, to evaluate the potential role of contemporaneous exposure. Associations between prenatal fluoride exposure (MUF_{cr}) and GCI at 4 y old could not be adjusted for contemporaneous fluoride exposure because urine samples were not collected from children when the MSCA (from which the GCI is derived) was administered. Maternal bone lead measured by a 109-Cd K-X-ray fluorescence (KXRF) instrument at 1 mo postpartum, a proxy for lead exposure from mobilized maternal bone lead stores during pregnancy (Hu et al. 2006), was included in the model to test for the possible confounding effect of lead exposure during pregnancy. We focused on the subset of women who had patella bone lead values because these were found to be most influential on our previous prospective study of offspring cognition (Gomaa et al. 2002). Average maternal mercury level during pregnancy was also tested for being a potential confounder (Grandjean and Herz 2011). Mercury was measured as total mercury content in the subsample of women who had samples of archived whole blood samples taken during pregnancy with sufficient volume to be analyzed using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., Shelton, CT, USA) as previously described (Basu et al. 2014).

To address the potential confounding effect of socioeconomic status (SES) we conducted sensitivity analyses that adjusted our model for SES (family possession score). The socioeconomic questionnaire asked about the availability of certain items and assets in the home. Point values were assigned to each item, and SES was calculated based on the sum of the points across all items (Huang et al. 2016). Given that the calcium intervention theoretically could have modified the impact of fluoride, in examining our results, we repeated the analyses with and without the Cohort 3 participants who were randomized to the calcium intervention to omit any potential confounding effect of this intervention. Another sensitivity test was performed to examine the potential effect of the psychologist who performed the WASI test by including tester in the regression model. The information about psychologists who performed the WASI was available for 75% of participants, as recording this data was

Table 3. Distributions of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and offspring cognitive scores across categories of main covariates.

	GCI Analysis					IQ Analysis				
Covariate	*n*	MUF_{cr}[a]	*p*-Value	GCI (Age 4)	*p*-Value	*n*	MUF_{cr}[a]	*p*-Value	IQ (Age 6–12)	*p*-Value
Mothers										
Age										
≥25 y	123	0.88 ± 0.36	0.45	96.22 ± 14.12	0.50	88	0.89 ± 0.37	0.98	95.75 ± 11.64	0.80
<25 y	164	0.92 ± 0.36		97.37 ± 14.43		123	0.89 ± 0.35		96.15 ± 10.76	
Education										
<12 y	153	0.91 ± 0.4	0.92	94.22 ± 14.23	0.001	111	0.87 ± 0.37	0.53	93.09 ± 10.54	<0.001
12 y	97	0.89 ± 0.34		98.56 ± 14.46		70	0.93 ± 0.35		98.29 ± 10.72	
>12 y	37	0.89 ± 0.42		103.49 ± 11.21		30	0.85 ± 0.31		101.3 ± 11.16	
Marital status										
Married	201	0.90 ± 0.37	0.81	96.40 ± 14.46	0.39	62	0.90 ± 0.35	0.79	96.55 ± 11.06	0.63
Other	86	0.91 ± 0.33		98.00 ± 13.88		149	0.88 ± 0.36		95.74 ± 11.16	
Smoking										
Ever smoker	141	0.90 ± 0.36	0.80	97.77 ± 13.9	0.30	102	0.90 ± 0.36	0.56	97.21 ± 10.7	0.12
Nonsmoker	146	0.91 ± 0.35		96.01 ± 14.63		109	0.87 ± 0.35		94.83 ± 11.41	
HOME score [b]										
Mid-low ≤30	49	0.88 ± 0.37	0.47	90.73 ± 13.36	<0.001	32	0.87 ± 0.36	0.85	89.88 ± 8.45	0.011
High >30	137	0.92 ± 0.38		99.29 ± 14.61		92	0.88 ± 0.38		99.05 ± 11.65	
Maternal IQ										
Mid-low ≤85	116	0.95 ± 0.35	0.09	93.16 ± 15.04	<0.001	86	0.92 ± 0.36	0.23	91.26 ± 9.72	<0.001
High >85	171	0.87 ± 0.36		99.4 ± 13.21		125	0.86 ± 0.35		99.23 ± 10.87	
Children										
Sex										
Boy	127	0.94 ± 0.36	0.09	93.93 ± 13.98	0.002	95	0.96 ± 0.38	0.008	96.82 ± 12.02	0.32
Girl	160	0.87 ± 0.36		99.22 ± 14.12		116	0.83 ± 0.32		95.29 ± 10.31	
Birthweight										
≥3.5 kg	241	0.91 ± 0.36	0.57	96.52 ± 14.36	0.33	201	0.89 ± 0.36	0.88	95.66 ± 11.29	0.58
<3.5 kg	46	0.87 ± 0.35		98.76 ± 13.88		10	0.88 ± 0.34		97.38 ± 9.42	
Gestational age										
≤39 wk	192	0.90 ± 0.35	0.90	96.66 ± 14.23	716	146	0.89 ± 0.36	0.712	95.71 ± 11.62	0.65
>39 wk	95	0.90 ± 0.37		97.32 ± 14.46		65	0.88 ± 0.34		96.58 ± 9.91	
First child										
Yes	96	0.91 ± 0.38	0.75	99.97 ± 12.87	0.009	68	0.88 ± 0.36	0.91	97.00 ± 11.00	0.36
No	191	0.90 ± 0.35		95.32 ± 14.73		143	0.89 ± 0.36		95.50 ± 11.17	
CUF_{sg} [c]										
≥0.80 mg/L						112	0.86 ± 0.32	0.49	96.80 ± 11.16	0.37
<0.80 mg/L						77	0.90 ± 0.38		95.37 ± 10.31	

[a]Maternal creatinine-adjusted urinary fluoride (mg/L).
[b]Home Observation for the Measurement of the Environment (HOME) score, measured using the separate age-appropriate instruments pertaining to children of ≤5 y old; and children >5 y old.
[c]Child contemporaneous specific gravity–adjusted urinary fluoride (available at the time of each child's IQ test).

added later to the study protocol. We also re-ran models with exposure outliers included as a sensitivity step. Finally, we ran models that focused on the cross-sectional relationship between children's exposure to fluoride (reflected by CUF_{sg}) and IQ score, unadjusted; adjusting for the main covariates of interest; and adjusting for prenatal exposure (MUF_{cr}) as well as the covariates of interest.

Results

Flow of Participants

Of the 997 total mothers from two cohorts evaluated, 971 were eligible after removing mothers <18 y old. Of these 971, 825 had enough urine sample volume to measure fluoride in at least one trimester urine sample, and of these 825 participants, 515 participants had urine samples with previously measured creatinine values, enabling calculation of creatinine-adjusted urinary fluoride (MUF_{cr}) concentrations. Of these 515, 3 participants were excluded based on the 10 extreme outlier values identified for MUF_{cr} (see the "Methods" section, "Exposure Assessment" subsection) and not having any other MUF_{cr} values to remain in the analysis. Thus, we had a total of 512 participants (mothers) with at least one value of MUF_{cr} for our analyses (Figure 1).

Of these 512 mothers, 312 had offspring with outcome data at age 4 (i.e., GCI), and 234 had offspring with outcome data at age 6–12 (i.e., IQ). Of these, complete data on all the covariates of main interest (as specified in the "Methods" section) were available on 287 mother–child pairs for the GCI analysis and 211 mother–child pairs for the IQ analysis. A total of 299 mother–child pairs had data on either GCI or IQ, and 199 mother–child pairs had data on both GCI and IQ (Figure 1).

Number of Exposure Measures per Subject

In terms of repeated measures of MUF_{cr} across trimesters, of the 287 participants with data on GCI outcomes; 25 participants had MUF_{cr} data for all three trimesters (11 from Cohort 2A and 14 from Cohort 3), 121 participants had MUF_{cr} data from two trimesters (48 from Cohort 2A and 73 from Cohort 3), and 141 participants had MUF_{cr} data from one trimester (51 from Cohort 2A and 90 from Cohort 3). Of the 211 participants with data on IQ outcomes, 10 participants had MUF_{cr} data for all three trimesters (6 from Cohort 2A and 4 from Cohort 3), 82 participants had data from two trimesters (32 from Cohort 2A and 50 from Cohort 3), and 119 participants had data from one trimester (40 from Cohort 2A and 79 from Cohort 3).

Comparisons across the Cohorts

In terms of the mother–child pairs who had data on all covariates as well as data on either GCI or IQ ($n = 299$), the mean (SD)

Table 4. Multivariate regression models: unadjusted and adjusted differences in GCI and IQ per 0.5 mg/L higher maternal creatinine-adjusted urinary fluoride (MUF_{cr}).

	GCI			IQ		
Estimate	*n*	β (95%CI)	*p*-Value	*n*	β ± S.E (95%CI)	*p*-Value
Unadjusted	287	−3.76 (−6.32, −1.19)	<0.01	211	−2.37 (−4.45, −0.29)	0.03
model A[a]	287	−3.15 (−5.42, −0.87)	0.01	211	−2.50 (−4.12, −0.59)	0.01
Model A − HOME	138	−3.63 (−6.48, −0.78)	<0.01	124	−2.36 (−4.48, −0.24)	0.03
Model A + HOME	138	−3.76 (−7.08, −0.45)	0.03	124	−2.49 (−4.65, −0.33)	0.02
Model A − CUF_{sg}				189	−1.79 (−3.80, 0.22)	0.08
Model A + CUF_{sg}				189	−1.73 (−3.75, 0.29)	0.09
Model A − SES	188	−4.55 (−7.23, −1.88)	0.01	199	−2.10 (−4.02, −0.18)	0.03
Model A + SES	188	−4.45 (−7.08, −1.81)	0.01	199	−2.10 (−4.06, −0.15)	0.04
Model A − Pb	167	−5.57 (−8.48, −2.66)	<0.01	177	−3.21 (−5.17, −1.24)	<0.01
Model A + Pb	167	−5.63 (−8.53, −2.72)	<0.01	177	−3.22 (−5.18, −1.25)	<0.01
Model A − Hg	141	−7.13 (−10.26, −4.01)	<0.01	149	−4.59 (−7.00, −2.17)	<0.01
Model A + Hg	141	−7.03 (−10.19, −3.88)	<0.01	149	−4.58 (−6.99, −2.16)	<0.01
Model A − Ca	194	−3.67 (−6.57, −0.77)	0.01	136	−3.23 (−5.88, −0.57)	0.02

[a]Coefficients from linear regression models adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked during the pregnancy vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Model A–HOME, model A for subset of cases who have data on Home Observation for the Measurement of the Environment (HOME) scores (but the model did not include HOME score). Model A + HOME, model A for subset of cases with HOME score, adjusted for HOME score. Model A − CUF_{sg}, model A for subset of cases who have data on child contemporaneous specific gravity–adjusted urinary fluoride CUF_{sg} (but the model did not include CUF_{sg}). Model A + CUF_{sg}, model A for subset of cases with CUF_{sg}, adjusted for CUF_{sg}. Model A−SES, model A for subset of cases who have data on socioeconomic status (family possession measured by questionnaire of family possessions) (but the model did not include SES). Model A + SES, model A for subset of cases with SES data, adjusted for SES. Model A–Pb, model A for subset of cases who have data on maternal bone lead (but the model did not include maternal bone lead). Model A + Pb, model A for subset of cases with data on maternal bone lead, adjusted for maternal bone lead. Model A−Hg, model A for subset of cases who have data on maternal blood mercury (but the model did not include maternal blood mercury). Model A + Hg, model A for subset of cases who have data on maternal blood mercury, adjusted for maternal blood mercury. Model A − Ca, model A for subset of cases who did not receive the Ca supplement (they received the placebo).

values of creatinine–corrected urinary fluoride for the mothers was 0.90 (0.36) mg/L. The distributions of the urinary fluoride, outcomes (GCI and IQ), and additional exposure variables examined in our sensitivity analyses (maternal bone lead, maternal blood mercury, and children's contemporaneous urinary fluoride) across the three cohort strata (Cohort 3-Calcium, Cohort 3-placebo, and Cohort 2A) and all strata combined are shown in Table 1 for the mother–child pairs who had data for the GCI outcome ($n = 287$) and the IQ outcome ($n = 211$). The distributions showed little variation across the cohort strata except for bone lead and possibly blood mercury, for which, in comparison with Cohort 3, Cohort 2A clearly had higher mean bone lead levels ($p < 0.001$) and possibly higher blood mercury levels ($p = 0.067$). The mean (SD) values of specific gravity–corrected urinary fluoride for the children who had these measures (only available for those children who had IQ; $n = 189$) were 0.82 (0.38) mg/L.

In terms of the comparability of the participants across Cohort 2A and Cohort 3 with respect to our covariates, the distribution of the covariates was very similar with the exception of age of the offspring when IQ was measured, for which the mean ages were 7.6 and 10.0 y, respectively; and birth weight in the GCI analysis, for which Cohort 3 participants were slightly heavier than Cohort 2 participants (see Table S1).

GCI versus IQ Scores

There was a significant correlation between GCI at 4 y and IQ at 6–12 y old (Spearman $r = 0.55$; $p < 0.01$). There was no significant correlation between prenatal MUF_{cr} and offspring CUF_{sg} (Spearman $r = 0.54$, $p = 0.44$).

Comparisons of Participants in Relation to Missing Data

In comparing the participants who were included for the GCI and IQ analyses with the participants who were not included (based on data missing on GCI, IQ or other covariates), the distribution of covariates were similar except for sex, for which the proportion of females was somewhat higher in the included versus excluded group for both the GCI and IQ analyses (Table 2).

In terms of the sensitivity analyses, for each sensitivity variable of interest, we compared participants who had data on our exposures, outcomes, covariates, and the sensitivity variable of interest (and were thus included in the sensitivity analysis) versus participants who had data on the sensitivity variable of interest but were missing data on the exposure, outcomes, and/or covariates of interest (and were thus excluded from the sensitivity analysis; Table 2). It can be seen that for each sensitivity analysis, most of the participants with data on the sensitivity variable of interest also had data on the exposures, outcomes, and covariates and were therefore included in the sensitivity analysis. In addition, the distributions appeared to be similar comparing those included with those excluded in each sensitivity analysis (means were within 10% of each other), with the exception of maternal blood Hg, for which the mean levels for those included were 28.5% and 24.9% higher than the mean levels for those excluded in the GCI and IQ analyses, respectively.

Comparisons of GCI and IQ across Covariates

Table 3 shows mean and SD values for MUF_{cr} and offspring cognitive scores across categories of the covariates. In the participants with GCI data, the offspring cognitive scores were higher among mothers with higher levels of education, measured IQ, and HOME scores for both analyses; and scores were higher among first children and girls. In the IQ analysis a statistically significant difference was observed in MUF_{cr} as a function of child sex. No significant differences in MUF_{cr} values across levels of other covariates were observed. A modest difference (not statistically significant), was observed in MUF_{cr} as a function of maternal IQ ($p = 0.09$), and MUF_{cr} as a function of child sex ($p = 0.09$). Among other co-variates there were significant differences in age ($p < 0.01$) in both analyses.

Regression Models of GCI

Before adjustment, a 0.5 mg/L increase in MUF_{cr} was negatively associated with GCI at 4 y old [mean score −3.76; 95% confidence interval (CI): −6.32, −1.19] (Table 4). The association was somewhat attenuated after adjusting for the main covariates

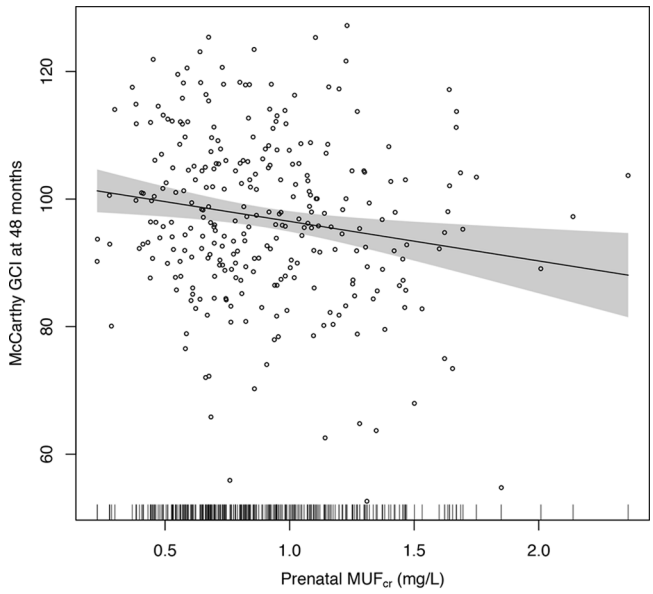

Figure 2. Adjusted association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and General Cognitive Index (GCI) scores in children at age 4 y. Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observations, $n = 287$.

(model A, −3.15; 95% CI: −5.42, −0.87). The smooth plot of the association between GCI and maternal prenatal urinary fluoride from an adjusted GAM model suggested a linear relation over the exposure distribution (Figure 2).

Regression Models of IQ

A 0.5 mg/L increase in prenatal fluoride was also negatively associated with IQ at age 6–12 y based on both unadjusted (−2.37; 95% CI: −4.45, −0.29) and adjusted models (−2.50; 95% CI: −4.12, −0.59) (Table 4). However, estimates from the adjusted GAM model suggest a nonlinear relation, with no clear association between IQ scores and values below approximately 0.8 mg/L, and a negative association above this value (Figure 3A). There was a nonsignificant improvement in the fit of the model when a quadratic term was added to the linear model ($p = 0.10$).

Sensitivity Analyses

In sensitivity analyses, adjustment for HOME score increased the magnitude of the association between MUF_{cr} and GCI, though the difference was less pronounced when associations with and without adjustment for HOME score were both estimated after restricting the model to the subset of 138 children with HOME score data (Table 4). The association of IQ scores with MUF_{cr} did not substantially change after adding HOME score to the model (Table 4).

The association between MUF_{cr} and IQ was attenuated slightly after adjusting for contemporaneous children's urinary fluoride (CUF_{sg}) and comparing estimates with [−1.73 (95% CI: −3.75, 0.29)] and without [−1.94 (95% CI: −4.15, 0.26)] adjustment for CUF_{sg} among the 189 children with this data (Table 4). In addition, the evidence of nonlinearity was more pronounced, with no clear evidence of an association for MUF_{cr} <1.0 mg/L

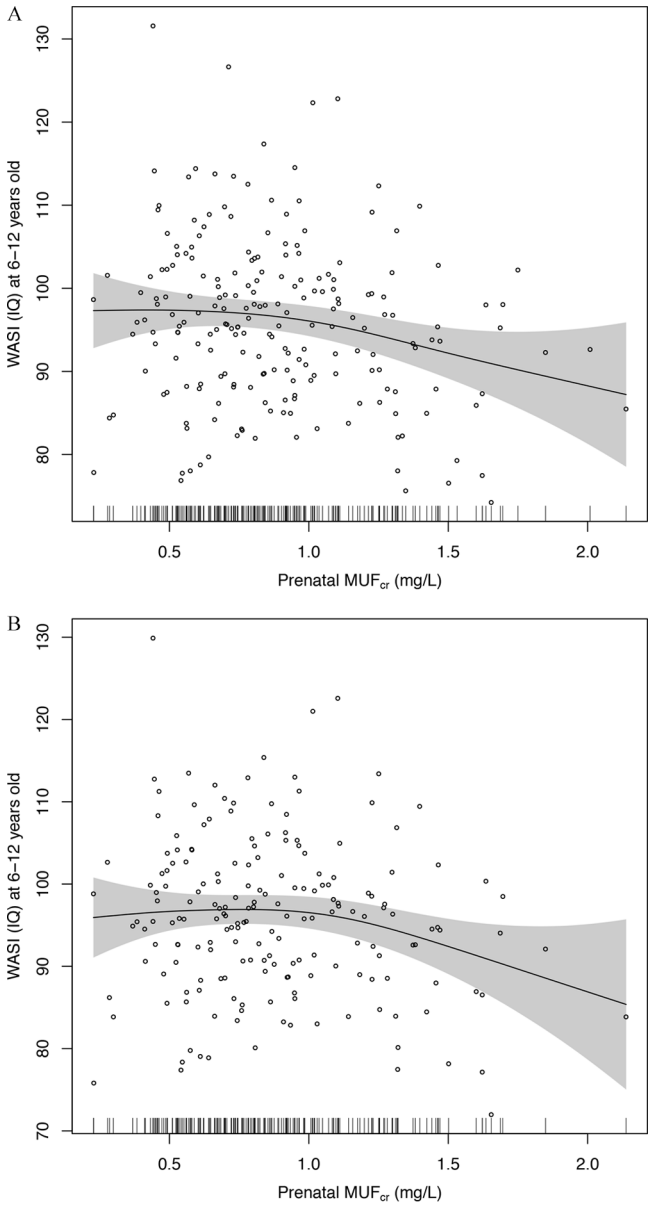

Figure 3. (A) Adjusted association of maternal creatinine-adjusted urinary fluoride (MUF_{cr}) and children's IQ at age 6–12 y. Adjusted for gestational age, weight at birth, sex, parity (being the first child), age at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education, and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observation, $n = 211$. (B) Association of maternal creatinine-adjusted urinary fluoride ($MUFU_{cr}$) and children's IQ at age 6–12 y, adjusted for specific gravity–adjusted child urinary fluoride (CUF_{sg}). Adjusted for gestational age, weight at birth, sex, parity (being the first child), age and CUF_{sg} at outcome measurement, and maternal characteristics including smoking history (ever smoked vs. nonsmoker), marital status (married vs. others), age at delivery, IQ, education. and cohort (Cohort 3-Ca, Cohort 3-placebo and Cohort 2A). Shaded area is 95% confidence interval. Short vertical bars on the *x*-axis reflect the density of the urinary fluoride measures. Individual data points are individual observation, $n = 189$.

based on the GAM model (Figure 3B), and a significant improvement in model fit when a quadratic term was added to the linear regression model ($p = 0.01$).

When we restricted models to subsets of children with available data for each additional covariate, there was little difference

between adjusted and unadjusted associations between MUF_{cr} and GCI or IQ when socioeconomic status (family possession), maternal bone lead, and blood mercury, were added to models (Table 4). However, the effect estimates associated with MUF_{cr} for these analyses appear to be higher in the subsets with available data for these variables.

Adding tester (psychologist who performed WASI) in the model did not substantially change the results (data not shown). In the sensitivity analyses in which we excluded Cohort 3 participants who received the calcium supplement, we continued to observe a negative association between MUF_{cr} and GCI [0.5 mg/L increase in MUF_{cr} associated with 3.67 lower GCI (95% CI: −6.57, −0.77), $n = 194$]; and between MUF_{cr} and IQ [0.5 mg/L increase in MUF_{cr} associated with 3.23-lower IQ (95% CI: −5.88, −0.57), $n = 136$].

In sensitivity analyses in which we re-ran models that included the 10 outliers with respect to fluoride exposure (for each of seven participants already in our models, an additional value of MUF_{cr} [from a different trimester]; for three participants, a value of MUF_{cr} that then allowed the participants to be added to our models), the results did not change in any meaningful way (data not shown). There were no outliers with respect to cognitive outcomes.

Independent Influence of Child Fluoride Exposure

Finally, in models that focused on the cross-sectional relationship between children's exposure to fluoride (reflected by their specific gravity–adjusted urinary fluoride levels) and IQ score and that contained the main covariates of interest, there was not a clear, statistically significant association between contemporaneous children's urinary fluoride (CUF_{sg}) and IQ either unadjusted or adjusting for MUF_{cr}. A 0.5 mg/L increase in CUF_{sg} was associated with a 0.89 lower IQ (95% CI: −2.63, 0.85) when not adjusting for MUF_{cr}; and 0.77-lower IQ (95% CI: −2.53, 0.99), adjusting for MUF_{cr} ($n = 189$).

Discussion

In our study population of Mexican women and children, which accounted for two of the three cohorts included in the ELEMENT study, higher prenatal exposure to fluoride (as indicated by average creatinine-adjusted maternal urinary fluoride concentrations during pregnancy) was associated with lower GCI scores in children at approximately 4 y old, and with lower Full-Scale IQ scores at 6–12 y old. Estimates from adjusted linear regression models suggest that mean GCI and IQ scores were about 3 and 2.5 points lower in association with a 0.5 mg/L increase in prenatal exposure, respectively. The associations with GCI appeared to be linear across the range of prenatal exposures, but there was some evidence that associations with IQ may have been limited to exposures above 0.8 mg/L. In general, the negative associations persisted in sensitivity analyses with further adjustment for other potential confounders, though the results of sensitivity analyses were based on subsets of the population with available data.

Overall, our results are somewhat consistent with the ecological studies suggesting children who live in areas with high fluoride exposure (ranging from 0.88 to 11.0 mg/L fluoride in water, when reported) have lower IQ scores than those who live in low-exposure or control areas (ranging from 0.20 to 1.0 mg/L fluoride in water) (Choi et al. 2012) and with results of a pilot study of 51 children (mean age 7 y) from southern Sichuan, China, that reported that children with moderate or severe dental fluorosis (60% of the study population) had lower WISC-IV digit span scores than other children (Choi et al. 2015). A distinction is that our study, which was longitudinal with repeated measures of exposure beginning in the prenatal period, found associations with respect to prenatal fluoride exposures.

To our knowledge, the only other study that is similar to ours was only recently published. Valdez Jiménez et al. (2017) studied the association of prenatal maternal urinary fluoride levels (not corrected for dilution) and scores on the Bayley Scales of Infant Development II among 65 children evaluated at age 3–15 mo (average of 8 mo). The mothers in their study had urinary fluoride levels of which the means at each of the three trimesters of pregnancy (1.9, 2.0, 2.7 mg/L) were higher than the mean MUF_{cr} in our participants (0.88 mg/L) (Valdez Jiménez et al. 2017). These levels of exposure were found to be associated with statistically significantly lower scores on the Bayley Scales' Mental Development Index (MDI) score after adjusting for gestational age, age of child, a marginality index, and type of drinking water (Valdez Jiménez et al. 2017). By comparison, our study had much longer periods of follow-up and larger sample sizes, controlled for a much larger set of covariates and sensitivity variables, and used creatinine–corrected urinary fluoride measures (which, by adjusting for urinary dilution effects, provides a more reliable measure of internal fluoride exposure).

With respect to understanding the generalizability of our findings to other populations, there are very few studies that measured prenatal fluoride levels among women derived from population-based samples. Gedalia et al. (1959) measured urinary fluoride in multiple samples collected from each of 117 healthy pregnant women living in Jerusalem, where fluoride in the water was approximate 0.50 mg/L, and reported mean levels per person that ranged from 0.29 to 0.53 mg/L. However, these analysis were not conducted utilizing modern analytical techniques. In a study of 31 pregnant women living in Poland, Opydo-Szymaczek and Borysewicz-Lewicka (2005) measured urinary fluoride in healthy pregnant women patients of a maternity hospital in Poland, where fluoride in the water ranged from 0.4 to 0.8 mg/L, and found a mean level of 0.65 mg/L for women in their 28th week of pregnancy, 0.84 mg/L in their 33rd week, and 1.30 mg/L in healthy non-pregnant women of similar age. This would suggest that the mothers in our study, who had a mean MUF_{cr} value of 0.90 mg/L, had fluoride exposures slightly higher than prior-mentioned populations.

In terms of comparing our findings with other studies of fluoride (using urinary fluoride as a biomarkers of exposure) and intelligence (i.e., those not involving prenatal exposures), of the 27 epidemiologic studies on fluoride and IQ reviewed by Choi et al. in their 2012 meta-analysis, only 2 had measures of urinary fluoride. Both were of urinary fluoride measures in children (not pregnant mothers), and neither corrected for dilution (either by correcting for urinary creatinine or specific gravity). Of these two, in comparison with the urinary fluoride levels of both our mothers (0.88 mg/L) and our children (0.82 mg/L), the mean levels of children's urinary fluoride were higher in the non-fluorosis (1.02 mg/L) and high-fluorosis (2.69 mg/L) groups found by Li et al. (1995) as well as the control (1.5 mg/L) and high-fluorosis (5.1 mg/L) groups described by Wang et al. (2007).

Among the limitations of our study are that we measured fluoride in spot (second morning void) urine samples instead of 24-hr urine collections. However, others have noted a close relationship between the fluoride concentrations of early morning samples and 24-hr specimens (Watanabe et al. 1994; Zohouri et al. 2006). Another limitation relates to the potential differences in the distribution of covariates over our study cohorts, raising the issue of potential bias. In the analyses we conducted across cohorts, we saw that, in comparison with Cohort 3, Cohort 2A clearly had

higher mean bone lead levels ($p < 0.001$) and possibly higher blood mercury levels ($p = 0.067$). However, we saw no other differences and the differences in these measures have a clear likely explanation: Cohort 2A had bone lead levels measured in 1997–2001 and Cohort 3 had bone lead levels measured in 2001–2005. Given that environmental lead and mercury exposures were steadily decreasing during this time interval (due to the phase-out of lead from gasoline), this difference likely relates to an exposure–time–cohort effect. We do not anticipate that this phenomenon would have introduced a bias in our analyses of fluoride and cognition controlling for bone lead.

Another limitation relates to the missing data that pertain to our covariate and sensitivity variables. In the comparisons of participants in relation to missing data (Table 2A,B), the proportion of females was somewhat higher in the included versus excluded group for both the GCI and IQ analyses, and the mean levels of maternal blood Hg for those included were 28.5% and 24.9% higher than the mean levels for those excluded in the GCI and IQ analyses, respectively. We also note that the coefficients for the associations between fluoride on cognition varied substantially in some of the sensitivity analyses, particularly with respect to the subgroups of participants who have data on SES, lead exposure, and mercury exposure (of which, for the latter, the effect estimates almost doubled). We do not have a ready explanation for this phenomenon, given that there is no obvious way that each of the selection factors governing which mothers had these measurements (discussed above) could have influenced the fluoride–cognition relationship. Nevertheless, it is not possible to entirely rule out residual confounding or in the population as a whole (that might have been detected had we had full data on larger sample sizes) or bias (should the subpopulations that had the data for analysis have a different fluoride–cognition relationship than those participants who were excluded from the analyses).

Other limitations include the lack of information about iodine in salt, which could modify associations between fluoride and cognition; the lack of data on fluoride content in water given that determination of fluoride content is not reported as part of the water quality monitoring programs in Mexico; and the lack of information on other environmental neurotoxicants such as arsenic. We are not aware of evidence suggesting our populations are exposed to significant levels of arsenic or other known neurotoxicants; nevertheless, we cannot rule out the potential for uncontrolled confounding due to other factors, including diet, that may affect urinary fluoride excretion and that may be related to cognition.

Another potential limitation is that we adjusted maternal urinary fluoride levels based on urinary creatinine, whereas we adjusted children's urinary fluoride levels based on urinary specific gravity; however, these two methods are almost equivalent in their ability to account for urinary dilution. We also had no data to assess the inter-examiner reliability of the testers administering the WASI test; however, the excellent reliability of these same testers in administering the McCarthy tests provides some reassurance that the WASI tests were conducted in a consistent manner.

Finally, our ability to extrapolate our results to how exposures may impact on the general population is limited given the lack of data on fluoride pharmacokinetics during pregnancy. There are no reference values for urinary fluoride in pregnant women in the United States. The Centers for Disease Control and Prevention has not included fluoride as one of the population exposures measured in urine or blood samples in its nationally representative sampling. The WHO suggests a reference value of 1 mg/L for healthy adults when monitoring renal fluoride excretion in community preventive programs (Marthaler 1999). As part of the NRC's review of the fluoride drinking-water standard, it was noted that healthy adults exposed to optimally fluoridated water had urinary fluoride concentrations ranging from 0.62 to 1.5 mg/L.

Conclusion

In this study, higher levels of maternal urinary fluoride during pregnancy (a proxy for prenatal fluoride exposure) that are in the range of levels of exposure in other general population samples of pregnant women as well as nonpregnant adults were associated with lower scores on tests of cognitive function in the offspring at 4 and 6–12 y old.

Community water and salt fluoridation, and fluoride toothpaste use, substantially reduces the prevalence and incidence of dental caries (Jones et al. 2005) and is acknowledged as a public health success story (Easley 1995). Our findings must be confirmed in other study populations, and additional research is needed to determine how the urine fluoride concentrations measured in our study population are related to fluoride exposures resulting from both intentional supplementation and environmental contamination. However, our findings, combined with evidence from existing animal and human studies, reinforce the need for additional research on potential adverse effects of fluoride, particularly in pregnant women and children, and to ensure that the benefits of population-level fluoride supplementation outweigh any potential risks.

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