Volume 31
Number 2
Pages 59-128
May 1998

FLUORIDE

JOURNAL

of the

International

Society for

Fluoride

Research

KAJ ROHOLM

THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

Vol. 31 No. 2 Pages 59-128 May 1998

FLUORIDE

QUARTERLY JOURNAL
OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

CONTENTS

Continued next page

BOOK REVIEW

DISCUSSION SECTION

DEATH KNELL FOR FLUORIDATION?

Two recent reports [1,2] that raise further questions about the alleged safety of water fluoridation reveal significant and disturbing brain cell neurotoxicity from relatively low concentrations of aluminium fluoride and sodium fluoride in drinking water of rats. Abstracted and commented on at length in this issue of FLUORIDE (pages 89-99), these investigations demonstrate that long-term ingestion by rats of drinking water containing either 0.5 ppm aluminium fluoride (AlF_3), or 2.1 ppm sodium fluoride (NaF) causes readily detectible damage not only to neuronal brain cells and vasculature but also to glomerular kidney cells.

In one set of experiments the commonly recommended 1 ppm fluoride ion level used in water fluoridation was present in the drinking water along with 0.5 ppm aluminium ion (reported as "0.5 ppm AlF_3"), and in the other series the same 1 ppm fluoride concentration was derived from 2.1 ppm NaF. Both brain cell and kidney cell abnormalities differed between the AlF_3 and NaF groups. Compared to controls with Al derived from the diet, the Al levels in the brain tissue were more than doubled in the AlF_3 group but not quite doubled in the NaF group. Although the kidney tissue Al levels were also doubled in the AlF_3 group, they were about the same in the NaF group.

Other previous experiments with rats showed that the toxicity of 0.5 ppm AlF_3 in the drinking water, including severe deterioration in overall health, was significantly greater than at 5 or even 50 ppm AlF_3. The reason for this paradoxical concentration effect is obscure, as is the mechanism by which aluminium along with fluoride passes through hydrophobic membranes and enters cells of the brain and kidneys.

Considering the clear-cut character of these laboratory findings, it is surprising that such adverse effects have not been reported long before now. After many years of supposedly intensive research, the 50th anniversary of the beginning of water fluoridation in Grand rapids, Michigan, was celebrated on 25 January 1995 as an occasion for "justifiable pride".[3] Even as recently as 1993 a National Research Council report of the US National Academy of Sciences concluded that the US Environmental Protection Agency's current Maximum Contaminant Level of 4 mg/L (4 ppm) for fluoride in drinking water was quite appropriate as an interim standard.[4] This position was supported by a selective review of data concerning dental and skeletal fluorosis, bone fragility, genotoxicity, carcinogenicity, and fluoride effects on the renal, gastrointestinal, and immune systems. Not cited, however, were fluoride effects on the central nervous system, including clinical reports of cognitive impairment[5] and findings of impaired intelligence in both animals[6] and humans[7,8] related to fluoride.

The "ethical basis"[9,10] for adding fluoride to water supplies depends on the procedure being safe or at least having a highly favourable benefit/risk ratio. The new findings reviewed here, showing impaired brain function from fluoride in conjunction with aluminium, have clearly tipped the balance against fluoridation. If we keep in mind the ancient dictum *primum non nocere*, then the death knell for fluoridation has begun to toll.

Bruce Spittle, Dunedin School of Medicine
Albert W Burgstahler, University of Kansas

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FLUORIDE-LINKED DOWN SYNDROME BIRTHS AND THEIR ESTIMATED OCCURRENCE DUE TO WATER FLUORIDATION

Kosei Takahashi*
Okegawa City, Saitama, Japan

SUMMARY: Down syndrome (DS) birth rates (BR) as a function of maternal age exhibit a relatively flat linear regression line for younger mothers and a fairly steep one for older mothers with the second line intersecting the first line a little above maternal age 30. Consequently, overall DS-BR for all maternal ages are not a very reliable parameter for detecting environmental influences, since they may be strongly affected by the ratio of the number of younger to older mothers. For this reason, data for mothers under age 30 were selected to detect an association between water fluoridation and DS for which the lower maternal age regression would be a much smaller contributing factor.

The early research of I Rapaport indicating a link between fluoride in drinking water and Down syndrome was followed by studies claiming there was no such association. Application of sound methodology to the data in those later investigations shows that none of the criticisms against Rapaport's work are valid. For example, in the data of J D Erickson on maternal age-specific DS births in Metropolitan Atlanta, Georgia, when the three youngest maternal age subgroups are reasonably combined into single groups for areas with and without water fluoridation, a highly significant association ($P < 0.005$) is revealed between fluoridated water and DS births.

It also appears that the dose-response line (DRL) of DS-BR for daily fluoride intake may have no allowable level that does not induce fluoride-linked DS births. Therefore fluoride may be one of the major causes of DS other than aging of mothers. The number of excess DS births due to water fluoridation is estimated to be several thousand cases annually throughout the world.

Key Words: Down syndrome; Down syndrome births; Fluoridation; Fluoride intake.

INTRODUCTION

In 1956-63 Ionel Rapaport,[1-3] a French-trained endocrinologist, working at the Psychiatric Institute of the University of Wisconsin, USA, presented evidence for a fluoride link to Down syndrome (DS, or "mongolism" – the trisomy-21 genetic disorder characterized by mental retardation, weak muscle tone, and epicanthic folds at the eyelids). Until recently, his data were compared with the mean values of Down syndrome birth rates (DS-BR) in fluoridated and non-fluoridated areas without consideration of the effects of demographic characteristics of the population.

In 1976 Erickson *et al* [4-5] (Centers for Disease Control, USA) discussed this problem in connection with their maternal age-specific DS incidence data in areas with and without fluoridated water (F and NF). Although they could recognize by inspection a higher incidence pattern of DS among younger mothers, they could not confirm the statistical significance of the difference between the two areas and concluded that there was no association between the incidence of DS and water fluoridation.

* Department of Internal Medicine and Biostatistics, Tokyo University Medical School, Tokyo, Japan (retired). Home address: Kosei Takahashi MD PhD, Kano 1788, Okegawa City, Saitama, 363-0001 Japan.

My first purpose is to reveal the truth possibly being concealed in their paper by correcting an inadequate insight into appropriate methodology.

SUBJECTS AND METHODS

For clarifying the distribution pattern of the mean values of DS-BR ascertained by the hospital records and/or multiple sources, data collected by Lilienfeld and Benesch[6] were examined.

The main data used here for detection of the effect of fluoride on DS-BR in the five-counties of the Metropolitan Atlanta, Georgia, area were ascertained from similar multiple sources.[4]

During his research on this problem, Professor A W Burgstahler, University of Kansas, obtained from Dr Erickson the observed sample sizes of the maternal age-specific subgroups which were not given in his paper. Professor Burgstahler kindly sent me these numerical data, and the analysis presented here was performed with them.

The NIS (National Intelligence Service) data in Table 2 in Erickson's first paper[4] and the 44 large US cities data in Table 2 in Erickson's second paper[5] were used for clarifying the distribution pattern of DS-BR ascertained from birth certificates.

The statistical method applied is the usual chi-square test for 2×2-fold tables. The data-processing rule, "Not to leave the subgroups unnecessarily overstratified,"[7] was applied which led me to combine the subgroups of young mothers in Erickson's Table 2[4] into one group so as to enhance the statistical power of the analysis.

The two exponential regression models behind the DS-BR-maternal age curve were obtained from publications by Jenkins[8] and by Lilienfeld and Benesch[6].

In my analysis of a DRL (dose-response line) between the daily intake of fluoride and the DS-BR, Heyroth's formula[9] was used to transform the concentration of fluoride in drinking water into the corresponding total daily intake, and then a linear regression analysis was performed.

RESULTS

Part I.

Quality of DS-BR data ascertained by birth certificates vs multiple sources

1. Amount of information provided by the two kinds of DS ascertainment

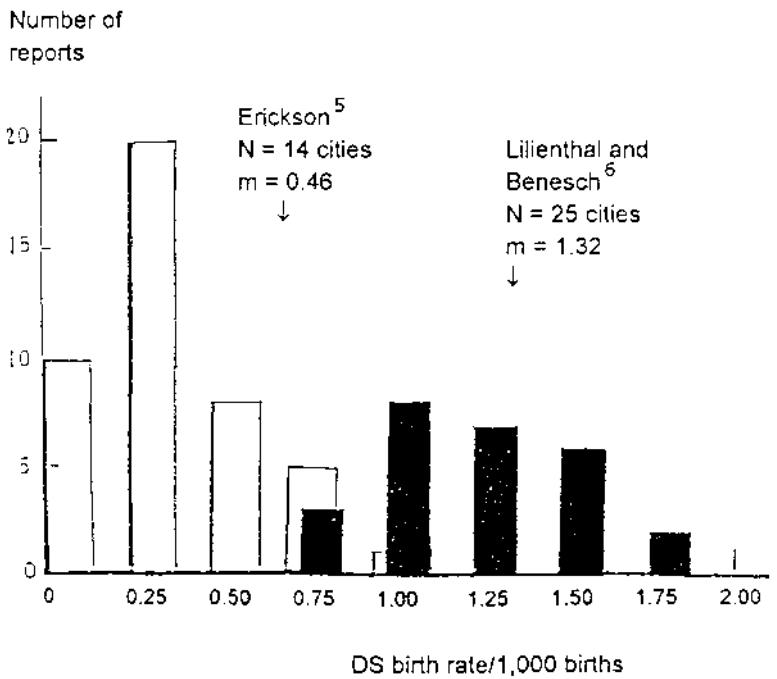
In 1969 Lilienfeld and Benesch[6] tabulated 34 studies on DS-BR (per 1,000 births) for the years 1923-1966. After eliminating six of the studies for lack of information on the method of ascertainment, the remaining 28 studies included two with ascertainment by birth certificates which gave very low rates of 0.32 and 0.52. Of the 26 studies ascertained by hospital records, one extraordinarily large rate of $X_N = 3.4$ was rejected as an unusual sample because the difference between the mean $\Delta = X_N - X$ corresponds to the rare probability of $P < 0.05$ as a random sample from this population. Finally the DS-BR from the-remaining 25 studies are shown as a histogram in the right side of Figure 1, which ranged from 0.83 to 1.90/1,000 births and gave 1.32/1,000 births as an algebraic average of 25 mean

values (Figure l). The data in about a half of these 25 studies were ascertained by hospital records and the others were further verified from multiple sources.

By contrast, Erickson *et al*[5] reported DS-BR ascertained by birth certificates in 44 large U.S. cities, among which 27 cities had a fluoridated water supply and 17 did not. The distribution of DS-BR in these cities, shown on the left side of Figure l, ranges from zero to 1.29/1,000 births and is located nearer the origin than those given by Lilienfeld[6]. The two distributions overlap only in several percent with one another. The algebraic average of 44 mean values was 0.456/1,000 births, only about one third that of Lilienfeld and Benesch. This low value of the total average is not influenced by the reported 50% sampling from birth certificates of sample size N in some of the cities, provided that the sampling was performed fairly randomly and the particular mean was obtained by dividing the total sum of the observed values N of their sample size by 2.

Therefore, DS-BR data ascertained by hospital records and/or multiple sources may be more useful than data ascertained by birth certificates alone. Whether the latter would be accompanied by reduction in quality will be discussed later.

Figure 1. Distribution of DS birth rate ascertained by birth certificates (left) and by hospital records (right) (K Takahashi 1997)



2. Two-component exponential regression model behind the DS-BR-maternal age curve

Jenkins in 1933 seems to be the first researcher who illustrated with four sets of data, DS-BR-maternal age curves on semi-logarithmic paper. He stated that the DS-BR behaved as a logarithmic function of the age of the mothers.

Over 30 years later, Lilienfeld and Benesch[6] reviewed 34 studies on DS-BR, among which only eight were stratified by quinquennial maternal ages. These eight are shown on a semi-logarithmic plot in Figure 2. These authors noted that the risk of DS remained practically constant up to 30 years, after which it continued to increase during the remainder of the reproductive period with the plots of the logarithms of the rates being almost linear. Practically, Lilienfeld and Benesch may be the first researchers who were aware of a double regression model behind the relation between DS-BR and maternal age.

Hence statistical analysis of DS-BR should be performed separately on younger mothers less than 30 (or 32 according to Jenkins) years of age and on older mothers more than 30 or 32 years of age.

Part 2.

Detection of fluoride-linked DS in Erickson's 1976 data for younger mothers

1. Data from Erickson's paper titled "Water fluoridation and congenital malformations: no association" [4]

These data were obtained primarily from the Metropolitan Atlanta Congenital Malformations Surveillance Program, which began in 1967, and were supplemented by the private survey data by Drs A J Ebbin and S Shimpler for the years 1960-1967.[4] All cases of DS were ascertained by regular staff visits to all hospitals that had obstetric or pediatric services, originally within one year after birth, and, supplemented later by a retrospective ascertainment, using multiple sources to track children born during 1960 to 1973. The DS-BR by maternal residence in areas with and without fluoridated water were recorded for five-year intervals of maternal age.

Table 1. DS birth rates, DS births, and total births for subgroups of specified maternal ages in F and NF areas of Metropolitan Atlanta, Georgia, 1960-1973

	F Area			NF Area		
Maternal age	DS birth rate*	No. of cases	Sample size	DS birth birth rate*	No. of cases	Sample size
≤19	7.7	19	24,811	3.8	7	18,319
20-24	6.9	41	59,266	4.0	15	37,612
25-29	6.8	34	49,865	4.1	11	26,884
	7.02	94	133,942	3.98	33	82,815

* per 10,000 white births

Figure 2. Incidence rates of Down Syndrome by maternal age from selected studies (Lilienthal and Benesch[6])

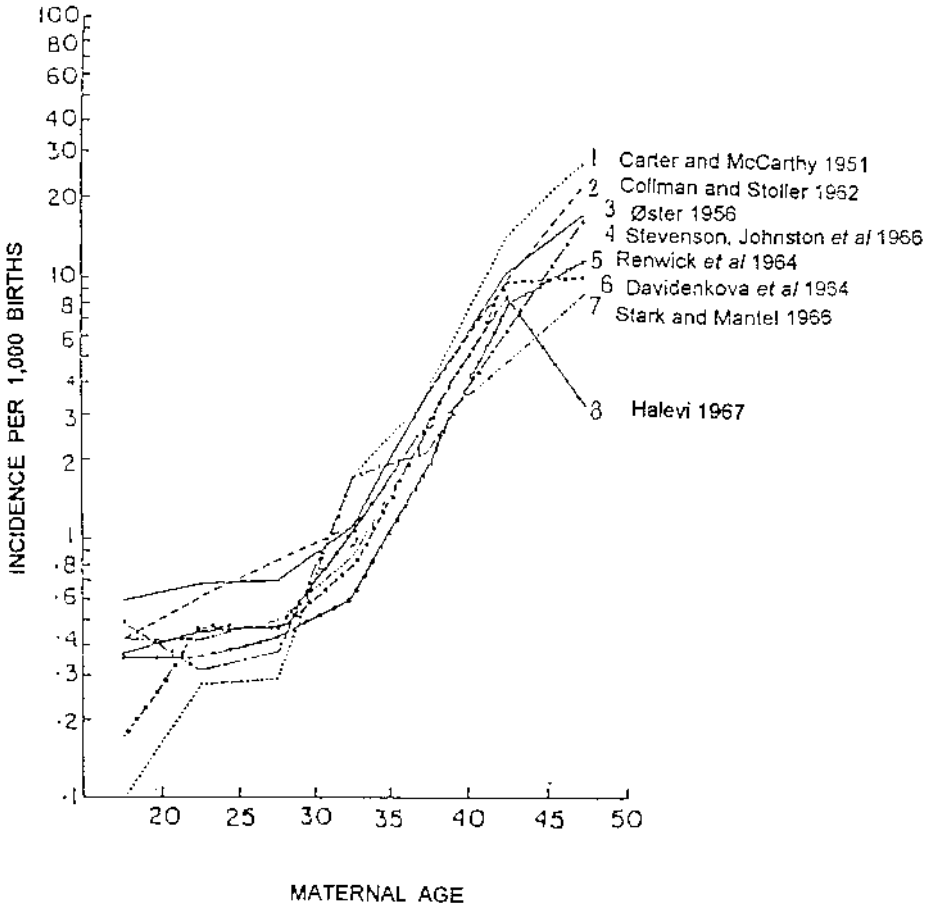


Table 2 . Significance test on DS-BR for subgroups of specified maternal ages in the F and NF areas of Table 1

Maternal	F Area			NF Area		
Age	DS +	DS -	Sum	DS +	DS -	Sum
# 19	19	24,792	24,811	7	18,312	18,319
20-24	41	59,225	59,266	15	37,597	37,612
25-29	34	49,831	49,865	11	26,673	26,884
Sum	94	133,848	133,942	33	82,782	82,815
	chi-square = 0.182 P > 0.50			chi-square = 0.021 P > 0.50		

The actual numbers of births for each maternal age interval, which were not given in Erickson's Table 2, were supplied by his reply to a request by Professor Burgstahler, and the total numbers were corrected to 166,186 for the fluoridated areas and to 101,639 for the nonfluoridated areas.

In his paper Erickson stated that the DS-BR of both data sets (Atlanta and NIS) suggested that in fluoridated areas, "there may be an increased incidence at young maternal ages". This statement shows that the possibility of an age-related fluoride link to DS was under consideration. Erickson tested the significance of the difference in DS-BR in the F and NF areas and obtained low chi-square values of 1.98, 2.93 and 1.78 for each of three younger maternal age subgroups, which seemed adequate for him to reject this hypothesis.

If Erickson had noted that the DS-BR in each of the young mother subgroups in the F and NF areas are nearly the same, he could then have considered whether to keep the three subgroups separate or to combine them into a single group according to the principle of data processing, *i.e.* "Not to leave the unnecessary over-stratification which prevents higher sensitivity of the statistical testing." [7]

Thus the preliminary procedure tried here was a chi-square test on the three DS-BR in each of the F and NF areas (Table 2). One thereby obtains very low chi-square values of 0.182 and 0.021, both of which are far from being significant. This result makes it reasonable to combine the three subgroups in each of the F and NF areas to obtain the four-fold array shown in Table 3.

The highly significant chi-square value of 8.04 calculated from Table 3 very clearly confirms the existence of fluoride-linked DS ($P < 0.005$), as Rapaport originally suggested in 1956.

Here it should be noted that Burgstahler, after he obtained the sample-size data for each subgroup from Erickson, confirmed the significant fluoride effect (chi-square = 6.8) in mothers less than 35 years of age but not in those less than age 40 in the Atlanta data, whereas not at all in the NIS data.[10,11]

The present analysis clarifies the theoretical basis for the empirical procedure by Rapaport and Burgstahler. Earlier, the present author,[12] who could not obtain a reply from Erickson, estimated the sample size for each maternal age subgroup by dividing the case number by the rate and confirmed the fluoride-linked DS by the same procedure given here.

Table 3. Number of DS births in combined young mother age groups from Table 1 specified by the F and NF areas

DS	F area	NF area	Sum
+	94	33	127
-	133,848	82,782	216,630
Sum	133,942	82,815	216,757
Rate /10, 000	7.02	3.98	

2. Loss of qualitative information in the data ascertained by the birth certificates

Although the problem of data quality is primary to the very essence of science, an appropriate set of data according to experimental design, if given, enables the statistician to deal with such a problem. Fortunately, Erickson[5,6] supplied such data but without appropriately addressing this problem.

Table 4 shows the influence of the data from the birth certificates (NIS and 44 cities, or "44C" data)[5] in comparison with those from the hospital records and/or multiple sources (Atlanta)[4] on DS-BR in F and NF areas.

The last column of Table 4 shows that the information on fluoride-linked DS seems to remain in the Atlanta data based on the hospital records and/or multiple sources, especially in those of young mothers. However, nothing remains in the NIS and 44C data based on only birth certificates, even in those of young mothers.

Table 4. Comparison of the DS-BR in the F and NF areas in three studies by Erickson

Item Survey	Maternal age	F DS/total birth	NF DS/total birth	F DS-BR*	NF DS-BR*	Δ = diff in DS-BR F and NF
Atlanta	All	166/166,182	86/101,639	10.0	8.5	1.5
	<30	94/133,942	33/82,815	7.0	4.0	3.0 †
NIS	All	115/234,300	524/1,032,100	4.9	5.1	-0.2
	<30	49/173,500	181/773,800	2.8	2.3	0.5
44C	All	178/432,580	90/204,185	4.1	4.4	-0.3
	<30	90/353,148	48/169,416	2.6	2.8	-0.2

* per 10,000 births † $P < 0.005$

This analysis thus presents evidence that, owing to loss of both quantitative and qualitative information, the data ascertained only by birth certificates has no power to negate a fluoride-link to DS birth.

Part 3. Daily fluoride intakes as one of the major causes of DS births

After we obtained a DRL (dose-response line) of DS-BR as a function of the concentration of F in drinking water, we wanted to estimate the contribution of food-borne fluoride in DS births.

For this purpose, the concentration of fluoride in the drinking water (x) must be first transformed into the total daily fluoride intake (z). In 1954 Heyroth[9] proposed a formula to do this. According to his formula, when x = 0 ppm, it will be transformed into z = 0.627 mg F/day and when x = 1.0 ppm, into z = 1.514 mg F/ day (Appendix 2).

Since fluoride other than in water fluoridation appears to be increasing year by year, Heyroth's 1954 formula may be better to apply in the USA to Erickson's survey data for 1960-1973 than any other newer formulae.

Now my task is the test of hypothesis "No allowable limit of fluoride in DS births" by the help of regression analysis, clarifying whether the dose-response line (DRL) goes through three points, namely two fluoride points $P_1(y_1\ z_1{:}\square)$, $P_0(y_0,z_0{:}\bigcirc)$ and the origin P(0,0:origin), shown in Figure 3.

As Erickson did not give the level of fluoride in the NF areas, we have to use a reasonable range for it: 0.1-0.3 ppm F. Figure 3 shows that the deviation of this DRL around the origin is less than 0.1 mg F/day on the z-axis and less than 1.3 DS/10,000 births on the y-axis.

If the estimates by Professor Burgstahler[11] starting from Rapaport's paper[2] are used (personal communication), we see that the DRL fits one theoretical and two observed points exactly without any recognizable deviation, as calculated in Appendix Table 1, in spite of the use of age-specific DS-BR for mothers under age 40 in Rapaport's study, not age 30 as in Erickson's work.

Though many environmental factors have been hypothesized for DS, *e.g.*, maternal illness, radiation exposure, viruses, endocrine factors, and others, the above analysis confirmed that there might be no other factor which promotes DS so steadily and widely as the total daily intake of fluoride except aging of mothers.

Part 4. Excess DS births linked to artificial water fluoridation worldwide

The DRL in Figure 3 enables us to estimate how many DS babies may be born to mothers ingesting 1 ppm fluoridated water. This estimate can be made as shown in Appendix 2. The number is around an additional 5-6 DS babies/10,000 births among young mothers living in fluoridated areas.

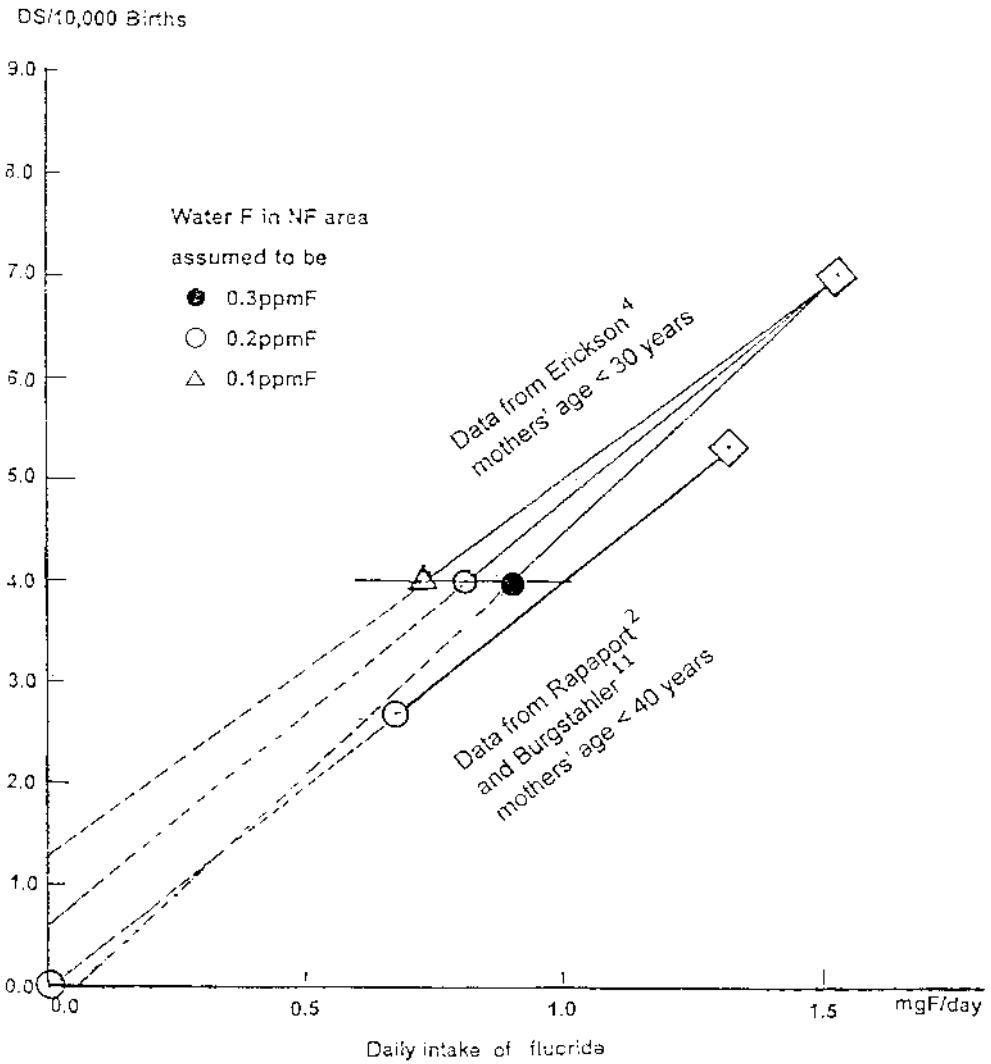
As Erickson's Atlanta data reported 7.02 cases of DS/10,000 births from mothers less than 30 years of age in the F areas and 3.98 cases in the NF areas, the excess DS births may be Δ = (7.02-3.98)/10,000 births ≈ 3.04 cases/10,000 births. Since 1990 FDI Basic Facts[13] reports that 325,000,000 people in the world are receiving artificially fluoridated water, an estimate of excess DS births linked to fluoridation worldwide may be derived from Erickson's data.

The first step is to estimate the number of births born to mothers under age 30 by multiplying the total birth rates in the countries by the percentage of births among young mothers. The second step is to adjust Erickson's lower DS-BR in comparison with those of Lilienfeld and Benesch[6] or Smith.[14] The third step is to include the increased daily intake of fluoride after Heyroth.[9]

As shown in Appendix 3, the number of DS babies linked to water fluoridation worldwide may thus be estimated to be about 3,000 cases per year (range 1,120-6,000). In any event, the excess DS births linked to artificial water fluoridation throughout the world may not be several tens but possibly several thousands annually, if fluoridation were to continue. From a humanitarian viewpoint such an enormous number of excess DS births linked to artificial water fluoridation is

not likely to be offset by a decrease in dental caries, since several communities in Japan, for example, now have less caries without use of any kind of fluoride.[15]

Figure 3. Two regression lines for DS birth rates among young mothers and the daily intake of fluoride (K Takahashi 1997)



DISCUSSION AND CONCLUSION

In 1975 Burgstahler[16] published an editorial review on DS in which he explained and supported Rapaport's papers written in French and countered the criticisms against Rapaport by Berry[17] and by Needleman *et al.*[18] In 1977 he presented his views to a committee hearing of the US House of Representatives.[19] This and his further research on this problem were also presented in Chapter 13 of the book *Fluoridation: The Great Dilemma*.[20]

In spite of Rapaport's extensive work and its subsequent validation, a recent Ad Hoc Subcommittee Report[21] published by the US Public Health Service dismissed Rapaport's research with the curt comment: "The results of three later studies conducted by other investigators with fuller ascertainment of cases did not confirm the finding." In my view the criticism of Rapaport in the Ad Hoc Subcommittee Report is completely meaningless from the viewpoint of methodology.

Lilienfeld and Benesch,[6] on the other hand, criticized Rapaport from the viewpoint of his low incidence of 0.44 DS cases per 1,000 births as proof of a low degree of ascertainment. But Rapaport accepted only cases ascertained and registered with a higher degree of accuracy by university and other research hospitals. The reduction in the number of cases due to such an advanced level of ascertainment is acceptable methodologically, provided the ascertainment was performed independently of whether the particular birth occurred in the F or NF areas.

Further, it should be noted that the DRL of the DS-BR in relation to the daily intake of fluoride according to Rapaport-Burgstahler's data[11] goes through the origin of the graph. This fact supports the accuracy of these data, despite the lower absolute values.

Taves criticized Burgstahler for his "selective use of data" (*i.e.*, use of young age maternal data). But the two-component exponential regression model behind the DS-BR-maternal age curve supports this use by Burgstahler and also by Takahashi (Figure 2).

The Ad Hoc Subcommittee Report[21] did not accept Rapaport's fluoride link to DS. However, it recommended studies to examine the reproductive toxicity of fluoride using various dose levels and the minimally toxic maternal dose. Further, it recommended studies to investigate whether fluoride is genotoxic or not. The report also stated that the US Public Health Service should sponsor scientific conferences to recommend the optimal safe level of total fluoride exposure from all sources combined (including drinking water).

Although the etiology of DS in areas with nearly a nil level of fluoride in the water supply has not been discussed until recently, my analysis reveals that fluoride from daily food may contribute to DS births. Supposedly, fluoride has been a steady environmental factor as well as an intrinsic aging factor in older mothers. Of course this interpretation will require another confirmation from various scientific disciplines. If the evidence becomes generally accepted, then fluoride might be an unavoidable public nuisance, especially for young mothers.

"The lesser is better" may be an important principle of life science in connection with fluoride.

Artificial water fluoridation for prevention of dental caries was introduced in 1945 in the USA and has been recommended and promoted by the World Health Organization. But now we must ask the people of the world whether such an amount of fluoride linked to Down syndrome can justify a possible decrease in dental caries or not. It must be stressed that the prevention of dental caries can be achieved by environmentally safer and less costly alternative procedures.[15]

ACKNOWLEDGEMENT

I thank Professor A W Burgstahler who kindly sent me large amounts of numerical data. I am also grateful for all his correspondence with me that greatly helped my methodological approach to this problem. I am also grateful to Dr Toru Murakami, former President of the Japanese Society for Fluoride Research, and Dr Kenji Akiniwa for their kind comments.

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APPENDICES

Appendix 1

Test of the hypothesis "No allowable limit of fluoride in DS births"

As Erickson did not give the level of fluoride in the NF areas, I have used a reasonable range of 0.1-0.3 ppm F. Figure 3 shows that the deviation of these dose response lines (DRL) around the origin is less than 0.1 mg F/day on the x-axis and less than 1.3 DS/10,000 births on the y-axis.

If the estimates by Burgstahler starting from Rapaport's paper for maternal age < 40 are used as data from a personal communication, we see that the DRL fits one theoretical and two observed points exactly without any recognizable deviation, as calculated in Appendix Table 1.

Appendix Table 1. Regression analysis on Rapaport-Burgstahler data

Nature of water F ppm	Geomet. mean of F ppm	(x) mg F per day	(y) DS/10,000 births
0.3-2.6	0.780	1.319	5.36
0.01-0.2	0.045	0.667	2.66
		0.000	0.00

$\Sigma x = 1.986$ $\Sigma y = 8.02$
Sx = 0.869918 (Sum of squares of x)
Sy = 14.365067 (Sum of squares of y)
Gxy=3.83482 (Product Sum of x and y)
(Water F ppm = 0 is substituted by 0.01 Which corresponds to the identification limit of F)

Factor	Analysis of Sy		Df †	F
Regr.	Cxy^2/Sz	14.363368	1	Fs=8454.41**
Remaind.	$Sy-Cxy^2/Sx$	0.001699	2-1=1	
Total	Sy	14	3-1=2	

** P < 0.01 $F^1_{,(0.05} = 161.4$, $F^1_{,(0.01)} = 4052$
† The degree of freedom of the origin is assumed to be 1

Appendix 2
Estimation of daily F intake from the concentration of F in drinking water (on the basis of Heyroth's work, 1954)

F F Heyroth in the USA[9] measured the daily amount of F ingested from food (y mg/day) in areas where drinking water contained F in 0.1 to 8 ppm (x). The contribution y showed a linear regression to x in the range of x = 0.1~6 ppm. From 2 liters of total daily use of water, two-thirds are obtained from foods or are used separately for cooking and the other one-third is drunk. The present author estimated the daily intake of F as the sum of y and 667 mL of x for each area specified by the concentration of F in drinking Water (Appendix Table 2).

Appendix Table 2. Estimated daily intake of F from food and F in drinking water (Heyroth and Takahashi)[9,12]

F in water (ppm)	F from food (mg/day)	F in 2L (mg)	F in 667mL (mg)	Daily Intake (mg/day)
0.0	0.627	0.0	0.000	0.627
0.1	0.649	0.2	0.067	0.716
0.2	0.671	0.4	0.133	0.804
0.3	0.693	0.6	0.200	0.893
0· 7	0.781	1.4	0.467	1.248
1.0	0.847	2.0	0.667	1.514
2.6	1.217	5.2	1.733	2.950

This table shows that 1-ppm water fluoridation increases the daily intake of F up to Δ = 1.514 - 0.617= 0.887 mg F/day which may correspond to the excess of 3.03 DS/10,000 births in young mothers (Erickson)[4]. Therefore a 1 mg increase of daily intake of F may correspond to a DS- BR of 3.03 x 1/0.887 = 3.42. If adjusted to the DS-BR of Lilienfeld [5] or Smith [14], the excess DS-BR may be 5.34 or 6.05.

Appendix 3.
Estimation of excess DS births among young mothers linked to fluoridation worldwide

I. Population factors

People receiving artificially fluoridated water: N = 325,000,00 (FDI, 1990).[13]

Population defining factors:

a) birth rate/1,000 population: 10/1000 - 15/100 - 20/1000

b) births from young mothers/total births: 0.75 - 0.80 - 0.85

c) Births from young mothers: n = (N × a × b)/year

II. Fluoride-linked DS factors

Excess DS birth/10,000 young mothers:

7.02(F) - 3.98(NF) = 3.04/10, 000 young mothers

DS births defining factors:

a) Ascertainment adjusting factor (DS births/10,000 population)
adjusting to Lilienfeld: 13.2 (Lilienfeld)/8.46 (Erickson) = 1.56
adjusting to Smith: 15 (Smith)/8.46 (Erickson) = 1.77

b) Adjusting to the excess daily fluoride intake (Heyroth): $d_1/d_0 = d_1/0.627$ mg/day

Estimates of the number of DS births linked to artificial water fluoridation:

min = $325 \times 10^6 \times 10/1000 \times 0.75 \times 3.04/10,000 \times 1.56 \times 1.0$ = 1,160/year

med = $325 \times 10^6 \times 15/1000 \times 0.80 \times 3.04/10,000 \times 1.67^* \times 1.5$ = 2 970/year

max = $325 \times 10^6 \times 20/1000 \times 0.85 \times 3.04/10,000 \times 1.77 \times 2.0$ = 5, 950/year

* (1.56 + 1.77)/2 = 1.67

DEVELOPMENTS IN THE ANALYSIS OF FLUORIDE 1995-1997

M L Wen, N H Shi, Y Qin* and C Y Wang
Kunming, China

This biennial review is a continuation of the previous survey[1] and covers the literature of the analysis of fluoride from July 1995 to June 1997.

Electroanalysis

Several different lanthanide fluoride (LnF_3, Ln = Ce, Nd, Sm) crystals were grown and used for construction of fluoride ion-selective electrodes. Aspects of crystal growing, particularly the high-temperature dimorphism of SmF_3 were addressed, and the effects on the electrode resistance and response characteristics of crystal quality, doping, and membrane geometric dimensions were studied.[2] Hydroxide ion interference with three lanthanide fluoride single crystal membranes (LnF_3, Ln = La, Ce, Nd) has been examined and shown to be due to the formation of hydroxo-complexes formed within a gel layer at the membrane surface. A mechanism has been proposed for the response of the electrodes to hydroxide ion.[3]

Based on integration of a pervaporation process and potentiometric detection in a laboratory-made module, methods were developed for the determination of F^- in liquid and solid samples by formation of a volatile product with hexamethyldisiloxane. These methods were successfully applied to the determination of F^- in orange tree leaves, tap water, well water, fertilizers, and ceramic industry waste water.[4,5] Procedures for the determination of F^- in plant material employing acid digestion and solution analysis by F-ISE (fluoride-ion selective electrode) were compared to alkali fusion using various plant materials. Owing to failure of the acids to liberate F^- bound strongly within silicate minerals in plant materials, acid digestion could be only used to determine labile F^- instead of total F in plant materials. With acid digestion procedures, F^- concentrations determined in solution using the F-ISE were sensitive to solution pH, even at solution pH values where complexation of F^- with H^+ could be discounted. Therefore, both the ionic strength and the pH of sample and standard solutions should be matched when determining F^- concentration using F-ISE.[6]

TISAB IV [disodium tartrate-tris(hydroxymethyl)methylamine-HCl] buffer was used to complex the Al ions, and NaOH was added to standard solutions to keep both the ionic strength and the Na^+ concentration constant as well as the pH. No hydroxide ion interference was detected even though the pH of the solution was 8.57. The total fluoride in aluminum fluoride and cryolite samples was determined by direct potentiometry under these conditions.[7] In order to analyze rain water continuously with a F-ISE, various buffer systems were examined for sensitivity enhancement. The sensitivity could be maximized at around pH 2.8 using the conventional acetate buffer. Sorensen's buffer (a mixture of glycine and hydrochloric acid, pH 2.8) was the most suitable for rain water analysis. The limit of linear response was 1×10^{-7} mol dm^{-3} and the detection limit was 1×18^{-8} mol dm^{-3} when the sample-to-buffer dilution ratio was 10:1.[8]

Department of Chemistry, Yunnan University, Kunming, Yunnan 650091, China.
Correspondence to Professor C Y Wang.
* Department of Chemistry, Baoshan Normal College, Baoshan, Yunnan 678000, China.

Both fluoride and molybdenum have been determined in the same serum sample. Fluoride was analyzed by direct potentiometry in a solution containing $(CH_2)_6N_4$ and HNO_3 at pH = 5.4 whereas molybdenum was determined by adsorptive voltammetry after the above mentioned solution had been nitrified with mixed acid containing HNO_3 and $HClO_4$.[9] In another clinical setting, the degree of fluorosis in people working in a fluoride-polluted environment has been ascertained by analysis of fluoride in human hair. The method involves the oxygen flash method for decomposing the hair sample and the addition of auxiliary combustible adhesive paper to the sample wrapped in filter paper before ignition, combined with the use of Gran's multiple fluoride-electrode-coupled minicomputer.[10]

Based on the principle that enzymatic catalytic decomposition of hydrogen peroxide is inhibited by fluoride, a catalase biosensor has been developed to detect the substrate hydrogen peroxide and the inhibitors fluoride and cyanide in a phosphate buffer. The enzyme which catalyzes the decomposition of hydrogen peroxide to oxygen and water, was immobilized in a membrane by entrapment in polyacrylamide in contact with a Clark-type oxygen electrode. The F^- detection limit was 1 mg/L.[11] A simple, accurate and selective method has been described for determining morphine in illicit powders, based on monitoring the initial rate of fluoride ion liberated from the reaction of morphine with 1-fluoro-2,4-dinitrobenzene at pH 9 and 35°C with a solid-state fluoride ion-selective electrode.[12] Using affinity binding of the glucoenzymes peroxidase and glucose oxidase on pF-sensitive field-effect transistors ($Si/SiO_2/Si_3N_4/LaF_3$ layers), Koeneke *et al* have developed new reloadable biosensors. The basic measuring principle of these ISFETs is the change in current in response to the concentration of F^- ions. The immobilized glucoenzyme could be removed from the enzymatically inactive C or A basic membrane, and fresh enzymes could be bound again.[13]

Electroanalysis methods for fluoride are shown in Table 1.

Spectral Analysis

Fluoride as an inhibitor of the immobilized liver esterase (EC3.1.1.1), which catalyzes the hydrolysis of ethyl butyrate to ethanol and butyric acid, was determined spectrophotometrically in a flow injection system. To monitor the reaction a second enzymatic reaction was employed, in which alcohol dehydrogenase (EC1.1.1.1) immobilized on controlled pore glass catalyzed the oxidation of ethanol and reduction of the coenzyme NAD^+ to NADH, the latter being determined at 340 nm. There was a linear relationship between percent inhibition and fluoride concentration over the range $8x10^{-7} - 8x10^{-6}$ M (15-150 μgL^{-1}) fluoride. The relative standard deviation at mid-calibration range was below 4% (n = 5). The more common inorganic compounds present in water samples did not interfere in the fluoride determination.[14] Based on F^- inhibitory effect on the photo-oxidation of acridine catalyzed by iron(III), F^- has been determined by a flow injection spectrofluorometric method. A linear calibration curve was obtained over the range 0.76-9.5 μg/mL for F^-.[15]

Determination of fluoride by spectral analysis is summarized in Table 2.

TABLE 1. Determination of fluoride by electroanalysis

Method	Application	Reference
F-ISE with an extrapolation method	Continuous flow analysis of F^- in city tap water	*Electroanalysis* 7 (3) 221-224 1995
F-ISE	F^- in dentifrices	*Anal. Lab.* 4 (1) 47-50 1995
Electrochemical recognition	Selective recognition of F^- in the presence of other halides and common anions	*J. Chem.Soc.Chem Commun.* (3) 333-334 1995
F-ISE	F^- in diluted chromating solution	*Diandu Yu Tushi* 14 (1) 11-12 1995
F-ISE	F^- in solutions at low temperature	*Zavod Lab.* 61 (6) 1-4 1995
F-ISE with improved standard addition method	F^- in aqueous solution	*Nippon Kagaku Kaishi* (9) 743-745 1995
F-ISE	F^- in lanthanide matrix	*Anal. Lab.* 4 (2) 75-81 1995
F-ISE	F^- in alcoholic beverages and sugary solutions	*Ind. Bevande* 24 (138) 357-364 1995
F-ISE	F^- in drinking water	*Analyst* 120 (11) 2763-2767 1995
F-ISE	F^- in hexafluorosilicic acid	*Dopov. Nats. Akad Nauk Ukr.* (2) 97-100 1995
F/pH-ISE automatic analysis system	F^- in serum	*J. Autom. Chem.* 17 (6) 219-223 1995
F-ISE with ion exchange preconcentration-flow injection analysis	F^- in water	*Fenxi Huaxue* 23 (6) 671-673 1995
F-ISE	F^- in fluoridated milk	*Weisheng Yanjiu* 24 (6) 371-372 1995
F-ISE	F^- in natural water	*Guandong Weiliang Yuansu Kexue* 2 (8) 32-34 1995
Solid pH electrode combined with F-ISE	F^- in aqueous solution	*J Anal. Chem.* 51 (9) 892-895 1996
F-ISE	F^- in electroplating baths	*Rev. Acad. Cienc. Exactas, Fis., Quim. Nat. Zaragoza* 50 85-91 1995
F-ISE with sequential injection analysis	F^- in drinking water	*Electroanalysis* 8 (11) 1051-1054 1996
F-ISE with improved standard addition potentiometry	F^- in water/organic solvent mixtures	*Nippon Kagaku Kaishi* (2) 112-118 1997
ISE-FIA (flow injection analysis)	F^- in serum	*Biomed. Res. Trace Elem.* 7 (3) 125-126 1996
F-ISE	F^- In water	*Shuichuli Jishu* 22 (6) 342-344 1996
ISE	F^- in water	*Fushun Shiyou Xueyuan Xuebao* 16 (4) 21-25 1996

TABLE 2. Determination of fluoride by spectral analysis

Method	Application	Reference
Fluorescence quenching	F^- in hot spring water	*Fenxi Shiyanshi 14* (3) 30-32 1995
Graphite furnace molecular absorption spectrometry	F^- in oyster tissue and dental rinse	*Anal. Chim. Acta 315* (1,2) 167-176 1995
Spectrophotometric method	F^- in water	*Gaz. Woda Tech. Sanit 69* (8) 284-285 1995
Spectrophotometric or fluorometric method	F^- in aqueous solution	*Anal. Sci. 11* (2) 221-226 1995
Kinetic spectrophotometric method	F^- in soil, vegetables and water	*Huanjing Kexue 17* (4) 65-66 1996
FIA-Spectrophotometric method	F^- in water	*J. Flow Injection Anal. 13* (1) 35-43 1996
Alizarin complexone spectrometry	F^- in tea	*Iran Agric. Res. 14* (1) 111-117 1995
Spectrophotometry	F^- in dosage forms and dental preparations	*J. Pharm. Biomed. Anal 14* (8-10) 951-958 1996
Spectrophotometry	F^- in potable water	*Egypt. J. Anal. Chem. 5* (1) 31-39 1997
Spectrophotometry with Eriochrome Cyanine R indicator	F^- in water	*J. Chem. Educ. Software Ser. D 4D* (2) 60-63 82-84 1997
Extraction/spectrophotometry by a long capillary cell	F^- in natural water	*Fenxi Huaxue 25* (2) 201-204 1997

Chromatography

A carbon IC BI-02 column (4.6 mm id x 50 mm, Bio Tech Research) was installed in an ion chromatography unit (Dionex 4500i) equipped with an anion micromembrane suppressor (AMMS-MPIC) for simultaneous determination of a mixture of 8 anions (F^-, Cl^-, NO_2^-,Br^-, NO_3^-, SO_4^{2-}, HPO_4^{2-} and I^-). Calibration curves obtained from the peak areas of the 8 anions were linear with a high correlation coefficient (> 0.999) and a good relative standard deviation of 0.2-0.9% (n=10).[16] Methanol and dimethylformamide have been used as nonaqueous media for the separation of inorganic anions including fluoride. There were significant differences in separation selectivity with many anions showing reversed separation order compared to aqueous systems. Calibration of 11 inorganic anions separated in a phthalate electrolyte gave linear curves at $5x10^{-5} \sim 8x10^{-4}$ mol/L; detection limits were at 2.0×10^{-5} - 3.4×10^{-5} mol/L.[17] A pellicular anion-exchange column has been developed for determining inorganic anions including fluoride and oxyhalides such as chlorite, chlorate, and bromate. In contrast to conventional latex-agglomerated resins, this new anion exchanger allows the retention of fluoride well out of the water dip with elution of sulfate in <15 min using a carbonate-hydrogen carbonate eluent under isocratic conditions.[18]

Fluoride analysis by chromatography is summarised in Table 3.

TABLE 3. Analysis of fluoride by chromatography

Method	Application	Reference
Capillary ion analysis (CIA)	Deionized water used in nuclear power industry	*LC-GC 13* (2) 144-148 1995
Capillary electrophoresis (CE)	Drinking water	*Fenxi Huaxue 23* (3) 365 1995
Single column high pressure anion chromatography	Drinking water	*J. Liq. Chromatogr. 18* (7)1383-1403 1995
Ion chromatography (IC)	Organic rich anaerobic waters from peatlands	*J. Chromatogr. A 706* (1,2) 281-286 1995
CE	Rain water, river water and pond water	*Kankyo Kagaku 5* (2) 530-531 1995
Capillary zone electrophoresis (CZE)	Drinking and waste water	*Biomed Chromatogr. 9* (6) 281-282 1995
IC	water	*Anal. Sci 11* (6) 995-997 1995
IC	Drinking water	*Kogyo Yosui 445* 28-33 1995
IC	Slag of electric furnace	*Yejin Fenxi 15* (4) 36-37 1995
CZE	F^- in boric acid	*J. Chromatogr. A 716* (1,2) 311-317 1995
CE	River, rain, tap, and waste water	*Kankyo Seigyo 17* 49-55 1995
Non-suppressor type IC	F^- in aqueous solutions	*Int. J. Environ. Anal. Chem. 62* (3) 191-205 1996
Low-pressure IC	Acid rain	*Fenxi Shiyanshi 15* (1) 43-45 1996
IC	Drinking water	*Am. Environ. Lab. 8* (4) 30 32-37 1996
Low-pressure IC	Water and acid rain	*Huanjin Huaxue 15* (3) 273-277 1996
CZE	Toothpaste	*J. Chromatogr. A 734* (2) 416-421 1996
CE	Separation of F^-, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, HPO_4^{2-} and HCO_3^-	*Zheijiang Daxue Xuebao Ziran Kexueban 30* (2) 134-138 1996
IC	F^- in acids and concentrated inorganic salts	*Analusis 24* (2) 43-48 1996
Ion-exclusion-cation-exchange chromatography	Acid rain	*Trends Anal. Chem. 15* (7) 266-273 1996
HPLC	F^- in bricks after decomposition by melting	*GIT Fachz. Lab. 40* (8) 767-770 1996
Capillary GLC	F^- in dental creams	*Pharm. Acta Helv. 71* (4) 273-277 1996
IC with a novel stationary phase	Separation of F^- and other inorganic ions	*GIT Spez. Chromatogr. 16* (2)115-116 118-119 1996
CZE	Separation of F^- in toothpaste	*Anal. Commun. 34* (2) 67-68 1997
CE	F^- in rain water	*J. Chromatogr.A 734* (2) 416-421 1996

Miscellaneous methods for fluoride analysis are summarized in Table 4.

TABLE 4. Analysis of fluoride by miscellaneous methods

Method	Application	Reference
Microdiffusion	Ionizable F^- in cow's milk	*Analyst 120* (8) 2245-2247 1995
Shimadzu Ion Analyzer PIA-1000	F^- in rain water and river water	*Kankyo Kagaku 5* (2) 528-529 1995.
Proton microprobe	F^- distributions in mollusk shells	*Nucl. Instrum. Methods Phys. Res. Sect. B 104* (1-4) 333-338 1995
Continuous fluoride analyzer	HF emissions from ceramic ind. processes	*Tijdschr. Klei, Glas Keram.16* (8) 6-9 1995
Visual sensing utilizing a coupled redox reaction of ferrocenylboronic acids and dye molecules	F^- and saccharides	*Chem. Commun.* (3) 407-408 1996
Energy-dispersive X-ray analysis	Determination of fluorine-to-oxygen ratio	*Fresenius J. Anal. Chem. 356* (1) 37-40 1996
FIA	F^- in wastewater of aluminum plant	*An. Assoc. Bras. Quim. 45* (3) 106-110 1996

ACKNOWLEDGEMENT: We are grateful for support for this work from the Science Fund of Application and Foundation of Yunnan Province (Grant 96B008M) and the Science Fund of the Yunnan Education Committee (Grant 9512062).

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EFFECT OF FLUORIDE ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN GERMINATING MUNG BEAN SEEDLINGS

L G Wilde* and M Yu†
Bellingham, Washington, USA

SUMMARY: The influence of sodium fluoride (NaF) on superoxide dismutase (SOD) in mitochondria from germinating mung bean seedlings was studied. Mitochondrial preparations were obtained from seedlings treated daily with 0 (control), 0.1, 0.2, 1, and 5 mM NaF for 72 hours. NaF at 0.1 mM caused a small but significant increase in SOD activity, whereas 1 and 5 mM NaF decreased the enzyme activity by 10 and 40%, respectively.

Key words: Mung bean (*Vigna radiata*); Seed germination; Sodium fluoride; Superoxide dismutase (SOD).

INTRODUCTION

Fluoride has long been known as a potent metabolic inhibitor. Thus, fluoride can interfere with the metabolism of proteins, lipids, and carbohydrates.[1-4] Although the mechanism involved in the inhibition is not completely understood, fluoride often inhibits enzymes that require such cofactors as Ca^{2+}, Mg^{2+}, and Mn^{2+} ions. For example, NaF inhibited amylase[5] and invertase[6] in germinating mung bean (*Vigna radiata*) seeds. The inhibition is attributed, in part, to removal of the cofactor Ca^{2+}.[5,6] Changes in enzyme activity and intermediary metabolism caused by chronic fluoride exposure may lead to altered growth, development, and reproduction of the organism.

Among the many biochemical effects of fluoride, one that has attracted much recent attention is the generation of superoxide free radicals ($O_2^{-}\cdot$).[7] Superoxide free radical ($O_2^{-}\cdot$) is produced from O_2 by both natural and anthropogenic processes. It is produced naturally during mitochondrial respiration, upon exposure to UV-B radiation, and during an immune response by phagocytozing cells.[8-10] The anthropogenic processes of $O_2^{-}\cdot$ production are caused mainly by the action of various environmental pollutants such as NO_2, $CN^{\cdot}$, and the herbicides paraquat and nitrofen.[11-15]

Superoxide free radical is both an oxidant and a reductant and has the potential to cause adverse effects on biomolecules. For example, it can damage membrane lipids through lipid peroxidation and cause enzyme inactivation and DNA strand breakage.[16-18]

Living systems have evolved an intracellular enzymatic defense system to protect themselves against $O_2^{-}\cdot$. Superoxide dismutase (EC 1.15.1.1) (SOD) is an enzyme responsible for the breakdown of $O_2^{-}\cdot$. It is a metalloprotein and catalyzes the dismutation of $O_2^{-}\cdot$ to O_2 and H_2O_2.[19] By altering the concentration of $O_2^{-}\cdot$, SOD helps prevent both direct toxicity from $O_2^{-}\cdot$ and secondary toxicity from $\cdot OH$ and H_2O_2.

* Present address: 1124 Sudden Valley, Bellingham, WA 98226 USA. Correspondence to L G Wilde.

† Center for Environmental Science, Western Washington University, Bellingham, WA 98225-9181 USA.

A large volume of literature exists describing various physiological and biochemical effects of fluoride on higher plants. Observed symptoms in plants include depressed growth and development, chlorosis, decreased photosynthetic activity, necrosis, abscission of leaves, flowers, or fruits, impaired cone and seed production, and necrosis.[20] Fluoride has been shown to inhibit the activity of SOD. For example, *in vitro* studies with *E. coli* exposed to 30 mM fluoride showed a 50% reduction in SOD activity.[21] However, no information is available demonstrating *in vivo* inhibition of SOD activity by fluoride. We reported that exposure to 1.0 mM NaF inhibited mung bean germination, as manifested by decreased root elongation, altered tissue fatty acids[1] and soluble sugar composition.[6] In this communication, we report identification of SOD activity in mitochondrial preparations from mung bean seedlings and that NaF inhibited the enzyme *in vivo*.

MATERIALS AND METHODS

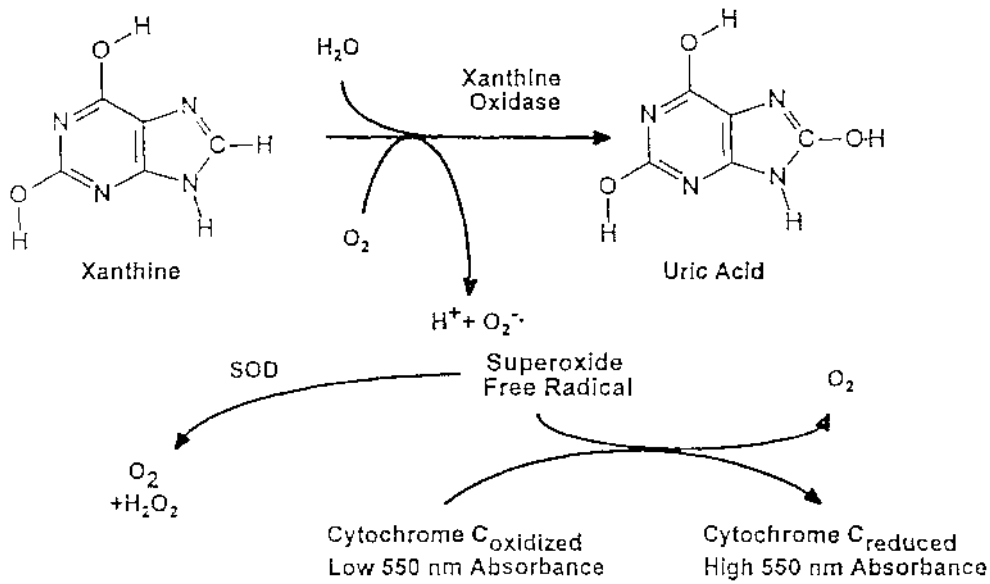
Seed germination and fluoride treatment: Mung bean seeds were germinated in an incubator at 25°C (±0.5°C). One-day-old seedlings were treated daily with 0 (control), 0.1, 0.2, 0.5, 1.0, or 5.0 mM NaF for 72 hours.

Preparation of crude extract: Crude extracts were obtained from whole seedlings or from the cotyledons, hypocotyls, and roots as needed. Extracts were prepared using the methods of Giannopolitis and Ries[21] with a minor modification. Mitochondrial fractions were prepared by differential centrifugation and the precipitated mitochondria were homogenized using a cold mortar and pestle, resuspended in 0.05 M KH_2PO_4-NaOH buffer (pH 7.8), and used in SOD assay.

Assay of SOD activity: The xanthine/xanthine oxidase assay used was a compilation of the methods from various sources.[19,23,24] Xanthine oxidase catalyzes the oxidation of xanthine to uric acid and in the process generates $O_2^{\cdot}$ (Figure 1). The $O_2^{\cdot-}$ production is coupled to the reduction of cytochrome C (a colorimetric reaction), which is followed spectrophotometrically, allowing for quantitative measurement. The SOD containing preparation added to the assay mixture will convert the superoxide free radical to H_2O_2 and O_2, therefore slowing the rate of cytochrome C reduction. During the assay, the absorbance was determined in a sprectrophotometer (IBM UV-Vis 9420) fitted with a Haake Temperature Regulator maintained at 29°C (±1°C). Additional data were obtained using an Hewlett Packard HP8452A Diode Array spectrophotometer using HP Kinetics software and regulated by an IBM 9550 Heating Cooling Fluid Circulator.

The assay mixture consisted of 2.90 mL solution (Solution A) containing 6.2 µg xanthine and 65.4 µg cytochrome C in 0.05 M KH_2PO_4-NaOH buffer (pH 7.8) and 50 µL enzyme. Prior to addition of the enzyme, the absorbance of Solution A at 550 nm was followed for 2.0 minutes at a 30-second interval to establish a baseline slope. The enzyme extract (50 µL) was then added to Solution A and

Figure 1. SOD assay reactions



absorbance measurement was continued every 30 seconds for another 4.0 minutes. The resultant slope from 2.5 minutes to 5.5 minutes was then compared to the slope of the first 2.0 minutes to determine if the test solution could reduce the rate of cytochrome C reduction (Absorbance at 550 nm [A_{550}] increase). Each sample was assayed at least twice, unless otherwise noted.

One unit of SOD activity was previously defined as a 50% decrease in the rate of cytochrome C reduction. Ideally, the initial rate of cytochrome C reduction before SOD addition was a change of 0.025 absorbance units per minute at 550 nm. To calculate the units of SOD activity in the assayed fraction, the rate of A_{550} increase from 2.5 to 5.5 minutes was divided by the initial rate from 0 to 2.0 minutes. This represents the percent difference in rate. This was subtracted from 100% to get the percent decrease in A_{550} increase. The resultant percent was then divided by 50% to normalize to units of SOD activity. These calculated units of SOD activity were then normalized for protein content by dividing by mg protein in the 50 μL sample assayed. Protein content of the sample was determined according to the method of Lowry *et al.*[25] using bovine serum albumin (BSA) as a standard.

Statistics: Each experiment was repeated at least three times with new groups of seedlings. Two or three subsamples were also run on each experimental unit to observe the extent of assay variability. Wilk-Shapiro tests were conducted to confirm that data fit a normal distribution. Equal variance within the data was

monitored using Bartlett's test of equal variances. Data that was not normally distributed or that did not have equal variance was log transformed. Randomized complete block style ANOVA tests were conducted, except where noted, with a $p < 0.05$ as the decision to reject or not reject the null hypothesis in each experiment. Asterisks on graphs denote statistical significance.

RESULTS AND DISCUSSION

Figure 2 shows the effect of varying *in vitro* pH (5.7, 6.4, 7.0, 7.4, 7.8, and 8.0) on mitochondrial SOD activity. No significant differences in the enzyme activity were observed within the pH range tested. However, the activity peaked at pH 7.8 and then sharply decreased. It appears that the optimum pH for the enzyme activity is at 7.8. This coincides with the optimum pH for SOD activity in peas and corn preparations reported by Giannopolitis and Ries.[26]

The effect of *in vitro* temperature changes on mung bean mitochondrial SOD activity was tested at 27, 30, 35, and 37°C. As shown in Figure 3, the enzyme activity increased with increase in temperature up to 37°C. Attempts to test the effect of temperature on mitochondrial SOD activity at 40°C were unsuccessful, because a baseline slope could not be established, possibly due to degradation of one or more components in the reaction mixture. It was apparent that even though activity could be maximized at 37°C, subsample variance also increased with increase in temperature. Thus, it can be concluded that using a lower assay temperature such as 30°C may provide more reproducible results.

The mitochondrial SOD activity increased with age of seedlings. The activity was very low in 2-day-old seedlings, but it increased nearly 8 and 20 times in 3-day-old and 4-day-old seedlings, respectively. SOD activity has been shown to increase with age of other test organisms. For example, total cytosolic and mitochondrial SOD activity in rat liver increased with age of the animal.[27] The activity in pea plumule greening and oat seed germination also exhibited a similar trend.[26] As growth continues during germination, the demand for respiration increases, so the number of mitochondria increases with age.

The enzyme activity differed within tissues, also. Based on the results of two separate experiments, it appears that the order of SOD activity in the tissues studied was hypocotyl>root>cotyledon (see Table).

TABLE. Mitochondrial SOD activity in cotyledons from seedlings exposed to NaF

NaF concentration (mM)	SOD activity (±SD)* (units/mg protein)
0.0	21.1 (±7.9)
0.1	21.7 (±6.1)
0.5	16.7 (±3.9)
1.0	18.8 (±3.2)

Values are the average of 3 determinations

Figure 2. Effect of pH on SOD activity

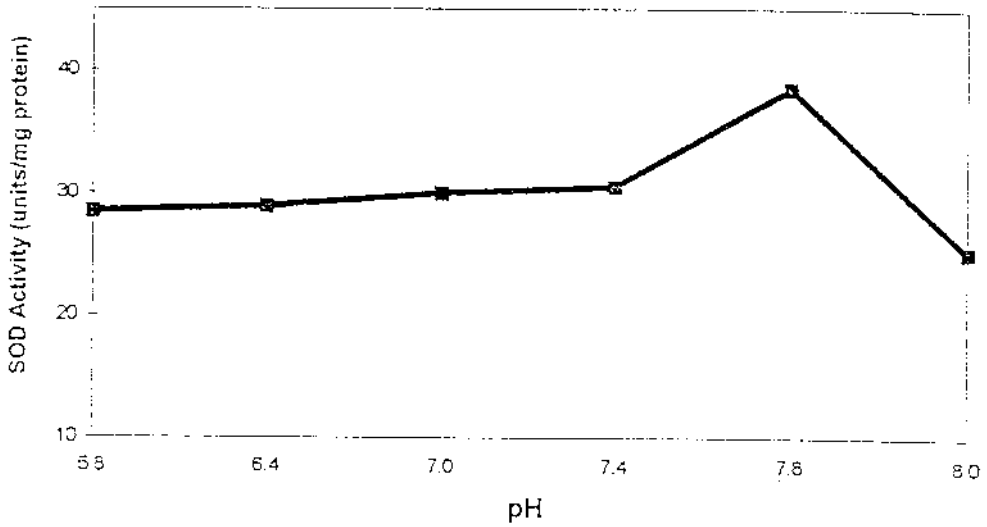


Figure 3. Effect of temperature on SOD activity

* indicates significantly different from each other (or from the control)

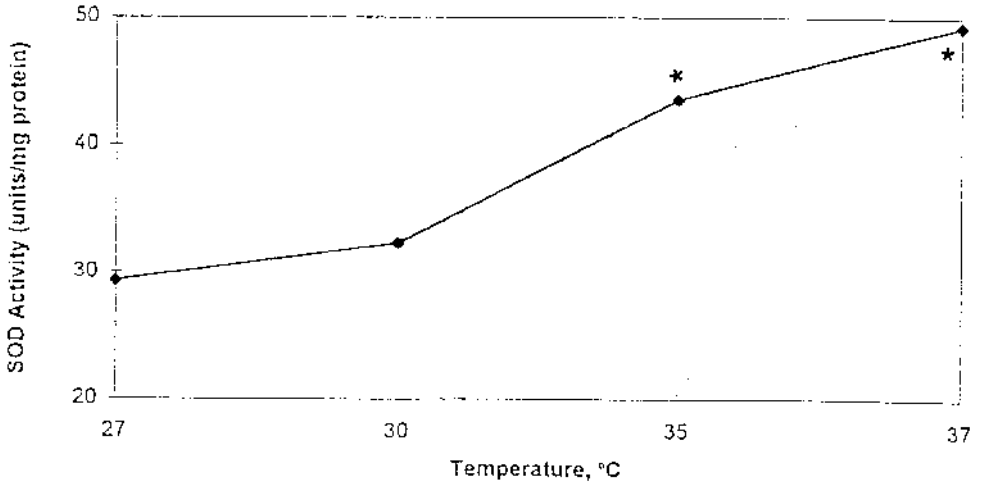
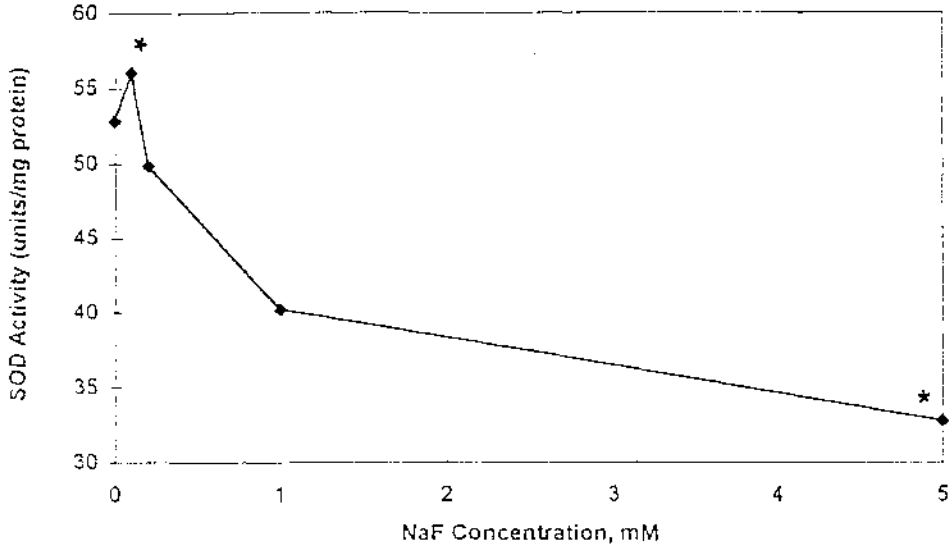


Figure 4. Effect of *in vivo* fluoride exposure on SOD activity in whole mung bean seedlings

* indicates significantly different from each other (or from the control)



Experiments were conducted to study the effect of *in vivo* fluoride exposure on SOD activity. For this purpose, seedlings exposed to varying concentrations of NaF for 72 hours were used to prepare the mitochondrial fraction. The results from experiments on mitochondrial preparations from whole seedlings are shown in Figure 4. A significant increase (10%) in enzyme activity occurred in seedlings exposed to 0.1 mM NaF. The activity was depressed steadily at 0.5, 1, and 5 mM NaF. In a separate set of experiments, the mitochondrial fraction was obtained from the cotyledons. The trend exhibited in these two sets of experiments was generally similar to each other. A slight increase in SOD activity in the 0.1 mM NaF group, and decreases in tissues treated with higher concentrations of NaF (see Table).

It is clear from the experimental results that treatment with NaF *in vivo* altered mitochondrial SOD activity in mung bean seedlings. It is interesting to note that NaF at low concentrations enhanced SOD activity, but depressed it at high concentrations (Figure 4). Although the mechanism involved in these changes is not clear, a similar trend has been observed in many toxicological studies.[28,29] The slight increase in SOD activity seen in seedlings treated with 0.1 mM NaF may be partly due to an increased metabolic activity or an increased SOD biosynthesis induced by fluoride exposure. Reiss *et al*[30] reported that, in rat brain and heart, inactivated SOD due to aging was replaced by new synthesis to maintain constant levels of the enzyme.

Acknowledgment: We thank the Bureau for Faculty Research and Huxley College, Western Washington University, for financial support. We also thank Terry Meredith, Clint Burgess, and Dr Don Williams for technical assistance.

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COMMENTS ON THE FINDING OF TOXIN-INDUCED BLOOD VESSEL INCLUSIONS AND ALTERATIONS IN NEURONAL AND CEREBROVASCULAR INTEGRITY FOLLOWING THE CHRONIC ADMINISTRATION OF ALUMINIUM FLUORIDE AND SODIUM FLUORIDE

Bruce Spittle
Dunedin, New Zealand

These two papers[1,2] (see abstracts on pages 91-99) document the development of significant pathology in the brains and kidneys of rats drinking water containing fluoride at a concentration of 1 ppm. What had previously been seen by some to be a safe procedure, the addition to water supplies of fluoride in a concentration of 1 ppm, has now been demonstrated to be associated with the occurrence of serious adverse effects in rats. However, rather than acting alone, fluoride acts in conjunction with aluminium to produce this toxicity.

Although it has sometimes been simplistically thought that the fluoride ion, at 1 ppm, has the same effect irrespective of what else is present with it, the fact is that the toxicity of fluoride is affected by the degree of hardness of the water reflecting the levels of magnesium and calcium. Jolly *et al*[3] found that, in four pairs of villages, the village in each pair with the higher level of magnesium hardness of the water had a lower skeletal fluorosis rate even though its mean water fluoride level was higher. High concentrations of dietary calcium and other cations that form insoluble complexes with fluoride can reduce fluoride absorption from the gastrointestinal tract.[4] Magnesium fluoride and calcium fluoride are relatively insoluble, dissociating less into their respective ions, so that for a given fluoride concentration they may be less likely to produce adverse effects than the same fluoride concentration in the form of sodium fluoride.

The authors of the present studies have found both aluminium fluoride and sodium fluoride can produce significant pathology in rats with points of both similarity and difference. It appears that a complex interaction may occur between fluoride and aluminium so that when both are present in rats' drinking water, at a low concentration, 0.5 ppm of trivalent aluminium ion and 1 ppm of fluoride ion, they combine to form aluminium fluoride, AlF_3, which has the ability to penetrate the blood-brain barrier and enter the brain, and also to become deposited in the kidneys. When 2.1 ppm of sodium fluoride is present in the drinking water of rats, corresponding to 1.1 ppm of sodium ion and 1 ppm of fluoride ion, the fluoride ion appears to be able to combine with aluminium from the food resulting in a similar entry to the brain and kidneys but with some points of difference. When the fluoride is present with larger amounts of aluminium, such as 5 ppm of trivalent aluminium and about 10 ppm of fluoride or 50 ppm of trivalent aluminium and about 100 ppm of fluoride, the resulting overt toxicity is less.

These papers are extremely important because of the severity of the pathology and the replication of the situation commonly occurring with the treatment of water for public consumption when both fluoride and alum, a double sulphate

of aluminium and potassium, is often added. Increased mortality occurred in the rats receiving 0.5 ppm of aluminium fluoride, containing 0.5 ppm of trivalent aluminium and about 1 ppm of fluoride, together with the deposition of increased amounts of aluminium in the kidneys, hypercellularity of the renal glomeruli, renal mesangial proliferation, and the deposition of protein in the renal tubules. The cerebral changes involved raised aluminium levels, damage to and loss of neurones, increases in β-amyloid, the presence of aluminium complexes in cerebral blood vessels, and of IgM in the neural parenchyma. The vascular inclusions were seen to be likely to cause a significant functional disturbance with decreased aerobic metabolism. The localization of the changes included the hippocampus which has a role in memory, the posterolateral area of the dorsal thalamus and the areas to which it is connected in the cerebral cortex. Similarly, the addition of 2.1 ppm of sodium fluoride, resulting in 1 ppm of fluoride, was seen to lead to analogous changes involving the kidney and brain. The cerebral effects included increased aluminium levels, IgM inclusions in the cerebral vasculature, increased IgM in the neural parenchyma, neuronal abnormalities, and increased β-amyloid deposition.

Although the precise manner in which aluminium exerts its toxic effects requires further study, the present papers demonstrate that cerebral and renal toxicity in rats can result from relatively low concentrations of aluminium and fluoride in the drinking water. The complexity of the problem is augmented by the finding that, although AlF_3 is lipid soluble and can pass through hydrophobic membranes, it is actually *more* toxic at 0.5 ppm trivalent Al and 1 ppm F^- than at higher concentrations such as 5 and 50 ppm Al^{3+} in association with 10 and 100 ppm F^-. This surprising fact suggested to the authors that fluoride at the higher concentrations might play a neuroprotective role against elevated cerebral accumulation of aluminium. The authors acknowledged, however, that the intrinsic toxicity of fluoride militates against proposing any such use, and the possibility that other, as yet unrecognized factors and mechanisms may be involved remains to be investigated. In any event, the results in these papers clearly point to the urgency for reassessing the risks of having both aluminium and fluoride present in drinking water.

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CHRONIC ADMINISTRATION OF ALUMINUM-FLUORIDE OR SODIUM-FLUORIDE TO RATS IN DRINKING WATER: ALTERATIONS IN NEURONAL AND CEREBROVASCULAR INTEGRITY

J A Varner, K F Jensen, W Horvath and R L Isaacson
Binghamton, New York, and Research Triangle Park, North Carolina, USA

Abstracted from *Brain Research 784* 284-298 1998

Objective

The question addressed in this study was to understand why 0.5 ppm of aluminum fluoride in the drinking water of rats was associated with a larger increase in infections and mortality than with higher concentrations of 5 ppm and 50 ppm of aluminum fluoride in the drinking water. This higher mortality had been found in an earlier experiment when rats were given 0.5 ppm, 5 ppm or 50 ppm aluminum for 45 weeks in their drinking water.

The earlier experiment had been done to help determine whether aluminum in drinking water might have a role in aging-related neurological impairments. The suggestion had been made that when both fluoride and aluminum were present in drinking water they would form fluoroaluminum complexes, such as AlF_3 in the stomach that, compared to their ionic forms, would be transported more easily into the blood stream and across the blood-brain barrier.

All the groups receiving AlF_3 had shown the same significant reduction in the number of cells in certain areas of the hippocampus relative to the control group. The level of aluminum in the brains was almost double that of the control group, while morphological abnormalities were observed throughout the brain and its blood vessels. Dramatic behavioral differences were not seen.

One possible reason for the increased infections and mortality of animals in the 0.5 ppm AlF_3 group was that the toxic agent of importance was the fluoride and that its absorption or its effects were antagonized by the higher levels of aluminum found with the higher doses of AlF_3. The present study was undertaken to compare 0.5 ppm of aluminum fluoride, AlF_3, with a comparable level of fluoride administered alone in the form of sodium fluoride.

Method

Twenty seven adult Long-Evans rats were administered one of three treatments for 52 weeks. All groups received double distilled deionized drinking water. No additives were present in water for the control group. One treated group received water containing 0.5 ppm AlF_3 (Al^{3+}) giving a fluoride concentration of about 1 ppm. The other treated group had water containing 2.1 ppm of sodium fluoride, to provide an equivalent amount of fluoride (about 1 ppm) as for the AlF_3 group.

Tissue aluminum levels of brain, liver, and kidney were assessed, and histological sections of brain were examined. For the morphological evaluations all counts and ratings were conducted by three individuals, two of whom were always blind to the treatment of the rats from whom the tissues came.

Results

No differences were found between the body weights of rats in the different treatment groups although more rats died in the AlF_3 group (5) than in the control group (1), $P=0.05$. A progressive decline in the appearance of the AlF_3 animals was noted throughout the experiment, with the hair becoming sparse and the yellowing which occurs with age. The skin became dry, flaky and of a copper color. The aluminum levels in samples of brain were higher in both the AlF_3 and NaF groups relative to the controls, $P<0.01$. The kidneys of the AlF_3 group had higher aluminum levels compared to both the control and NaF groups, $P<0.01$. Liver aluminum levels did not differ between groups.

The effects of the two treatments on cerebrovascular and neuronal integrity were qualitatively and quantitatively different with the alterations being greater in animals in the AlF_3 group than in the NaF group, and greater in the NaF group than in the controls. Examination of the distribution of the aluminum, using the Morin method for aluminum-fluorescence, showed the distribution in sections of brain to be exclusively associated with vasculature. This Al-fluorescence occurred in all three groups but there were consistent treatment-related differences in the intensity and extent of the aluminum-fluorescence. The NaF group had less Al-fluorescence associated with the vasculature than the control group, $P<0.03$. Renal changes were apparent in animals from both the NaF and AlF_3 groups. More monocyte infiltration was present in the kidneys of the AlF_3 group compared to the controls, $P<0.05$. No morphological abnormalities were present in the liver.

In the hippocampus, more moderately damaged and grossly abnormal cells were present in areas of the right hippocampus in the AlF_3 group than in the control group, $P<0.04$, $P<0.03$. In neocortical layers 2 and 3 of the right hemisphere, the AlF_3 group had more moderately damaged cells than the NaF group which had more normal cells, $P<0.04$. In neocortical layers 2 and 3 of the left hemisphere, the AlF_3 group had more abnormal cells than the controls, $P<0.02$. Corresponding increases in the frequency of normal appearing cells were observed in the neocortex of the control animals.

Neuronal density in the hippocampus was decreased in area CA3 in the left hemisphere of the AlF_3 group compared to the controls, $P<0.05$. No differences were found in layers CA1 and CA4 in either hemisphere. Neuronal density was decreased in neocortical layers 2 and 3 in the left hemisphere of the AlF_3 group compared to the controls and the NaF group, $P<0.05$. Neuronal density was also reduced in layers 5 and 6 of the left hemisphere of the AlF_3 group compared to the controls, $P<0.03$, $P<0.03$. No differences in neuronal density were found, in any of the groups, in layer 4 of the left hemisphere or in any of the layers of the cortex in the right hemisphere.

While in control animals the localization of IgM was largely restricted to the vascular lumen, indicating the integrity of the blood-brain barrier to IgM, in the AlF_3 and NaF groups staining for IgM in the neural parenchyma was increased in the right hemisphere, $P<0.03$, $P<0.01$. No differences were found among the groups in the left hemisphere. Minor amounts of IgM immunostaining were detectable in the hippocampus and dentate gyrus but no significant differences were present.

Differences in the amount of immunoreactivity for β-amyloid relative to that for amyloid A were most prominent in the vasculature of the dorsal thalamus. The control group had few instances of immunoreactivity while the tissue of animals from the AlF_3 group demonstrated a bimodal distribution of reaction product with either no reaction product staining or a high level. The AlF_3 group had more immunoreactivity for β-amyloid in the lateral posterior thalamic areas of both hemispheres relative to the controls, left $P<0.05$, right $P<0.01$. The NaF group differed from the control for immunoreactivity for β-amyloid in the right lateral posterior thalamic area with the controls having low reactivity and the NaF group having no or high levels of immunoreactivity, $P<0.01$.

Discussion and Conclusions

The high mortality rate in the animals receiving 0.5 ppm of aluminum in their drinking water found in the first study was replicated in this second study. Since the administration of sodium fluoride alone did not produce a similar mortality rate, this effect does not appear to be directly related to fluoride intake. The appearance of the AlF_3 animals, with sparse hair and a copper-colored hypermelanosis of the underlying skin, may be indicative of several diseases including chronic renal failure. Histological evidence of glomerular distortions was present in both the AlF_3 and NaF groups.

The overall aluminum level of the kidneys in the AlF_3 group was nearly twice that of the NaF group and possibly may have been associated with a greater level of physiological dysfunction. The kidney is critical to the elimination of both aluminum and fluoride, and these changes may have impaired the elimination of these elements, detoxification in general or homeostasis of other ions such as calcium.

Both the AlF_3 and NaF groups had increased brain aluminum levels relative to controls. The aluminum level in the NaF group was double that of controls and the aluminum level of the AlF_3 group even greater. The aluminum detected in the controls and NaF groups is most likely to have come from the rat chow where the reported levels of aluminum range from 150 ppm to 8300 ppm. Fluoride commonly occurs in food and water and is almost completely and quickly absorbed from the gastrointestinal tract. In the present experiment, the AlF_3 in the drinking water was prepared to form optimally a fluoroaluminum species capable of crossing the gut and vascular barriers. It is possible that the sodium fluoride-treated group was able to form some amount of an AlF_3 also capable of becoming bioavailable.

In general, the reduction of neuronal density in the neocortex of the left hemisphere was more prominent in the AlF_3 group than the NaF and control groups. Cellular abnormalities in the form of chromatin clumping, enhanced protein staining, pyknosis, vacuolation, and the presence of ghost-like cells were also more common in the AlF_3 group in the left hemisphere. Vascular Al-fluorescence was more pronounced throughout most structures of the left hemisphere of the AlF_3 group. The hippocampus was an exception to these findings with abnormalities being found only in the right hemisphere in the CA1 and CA4 areas of both the AlF_3 and NaF groups. The right hippocampus also had higher levels of aluminum-induced fluorescence than the left hippocampus.

Possible cellular mechanisms which might underlie the association between regional aluminum accumulation and regional patterns of neuronal injury include transferrin transport, calcium homeostasis and second messenger systems, alterations in neuronal cytoskeleton, and alterations in the cerebrovasculature. Transferrin transport carries certain metals, including aluminum, into the cells by a receptor complex located on plasma membranes. Neurotoxic reactions to aluminum may also result from disruption of ion homeostasis and second messenger systems, in particular G-proteins. The activation of G-proteins in turn initiates a chain of reactions beginning with adenylate cyclase, cAMP, and protein kinases that ultimately result in increased phosphorylation of various substrates. G-proteins are involved in the regulation of ion channels, metabolism, gene expression, and cytoskeletal structures via second messenger systems.

The deposition of aluminum in the cerebrovasculature was seen to have the potential also to contribute to the observed neuronal injury by altering the blood-brain barrier or cerebral blood flow. Both the AlF_3 and NaF groups had increased levels of IgM in the cortex of the right hemisphere compared to the controls. Serum proteins, such as IgM and other antibodies, are typically excluded from neuronal tissue by the blood-brain barrier. The increased immunoreactivity of IgM in the present study may be indicative of a compromise in the blood-brain barrier occurring possibly through changes in lipophilicity or the potentiation of existing transport mechanisms.

Striking parallels were seen between aluminum-induced alterations in cerebrovasculature and those associated with Alzheimer's disease and other forms of dementia where microvascular abnormalities display a regional and laminar specificity in accordance with neuronal degeneration patterns, suggesting that the alterations in the cerebrovasculature may be a primary event in neurodegenerative disease. Accumulation of β-amyloid has been found in the cerebrovasculature and spinal cord vasculature of elderly persons and may be a consequence of the development of blood vessel abnormalities. Vascular tissues produce β-amyloid and the vascular deposition of β-amyloid may result from alterations in the basement membrane of blood vessels that frequently occurs with ageing. Although it is uncertain whether β-amyloid is a causative factor in neurodegeneration or a secondary consequence of injury, aluminum appears to have the capacity to influence the distribution and properties of β-amyloid. In the present study animals in both the AlF_3 and NaF groups exhibited a bimodal distribution of vascular β-amyloid in both hemispheres in the lateral posterior thalamus being either absent or present in high levels in both groups. This increase in vascular β-amyloid in the lateral posterior thalamus may be causally related to the neuronal degeneration found in the area to which it is principally connected, the superior parietal cortex.

In contrast, the control group consistently had only low levels of immunostaining for β-amyloid, a unimodal distribution. While the presence of low levels of β-amyloid in the controls was understandable as being the possible result of a natural continuing, but limited, production of the protein throughout life, the bimodal distribution of β-amyloid in the AlF_3 and NaF groups was not understood.

While the present results do not address the causal mechanisms of aluminum-nduced neural degeneration, they do demonstrate that ingested aluminum eaches the brain, and in such animals there exists evidence of neural injury. Vhile the small amount of aluminum of 0.5 ppm AlF_3 in the drinking water of rats equired for neurotoxic effects was seen as surprising, the neurotoxic results of NaF at the dose given in the present study, 2.1 ppm or about 1 ppm of fluoride, was seen as even more so.

As dietary sources of fluoride are additive in animals it was considered that, for the animals in the NaF group, the fluoride in rat chow, such as Purina Rodent Laboratory Chow, together with their drinking exceeded tolerable levels. Fluoride has diverse actions on a variety of cellular and physiological functions including the inhibition of a variety of enzymes, a corrosive action in acid media, and the production of hypocalcemia, hyperkalemia and possibly cerebral impairment.

Few chronic toxicity studies of fluoride have included extensive histological characterization of injury to the brain but have usually been limited to weight loss, dental and skeletal changes, indicators of carcinogenesis, and damage to soft tissues. The results of the present study indicate that more intensive neuropathological evaluations of fluoride effects on the brain is likely to be of value.

In summary, the chronic administration of aluminum fluoride and sodium fluoride in the drinking water of rats resulted in distinct morphological alterations in the brain, including effects on neurones and the cerebrovasculature. Further studies of aluminum fluoride and sodium fluoride are needed to establish the relative importance of a variety of potential mechanisms contributing to the observed effects as well as to determine the potential involvement of these agents in neurogenerative diseases.

Key words: Aluminum-fluoride; Amyloid; Brain; Cerebrovasculature; Hippocampus; Neurotoxicity; Rat; Sodium fluoride.

Reprints: Julie A Varner, Lineberry Research Associates, PO Box 14626, Research Triangle Park, North Carolina 27709, USA. Fax: +1-919-547-0974.
E-mail: jvarner@lineberryresearch.com

TOXIN-INDUCED BLOOD VESSEL INCLUSIONS CAUSED BY THE CHRONIC ADMINISTRATION OF ALUMINUM AND SODIUM FLUORIDE AND THEIR IMPLICATIONS FOR DEMENTIA

R A Isaacson, J A Varner, and K F Jensen
Binghamton, New York, and Research Triangle Park, North Carolina, USA

Abstracted from *Annals of the New York Academy of Sciences 825* 152-166 1997

Objective: The questions being addressed involved the effects of aluminum fluoride and sodium fluoride on the brain. Until the aetiology of Alzheimer's dementia was better understood it was considered appropriate to investigate the possible contribution of aluminum to neurotoxicity. Following earlier experiments, a study was made of the effect of aluminum fluoride on the brain. After considering the results of this work a further experiment was carried out in which the effect on the brain of aluminum fluoride was again studied and, in addition, that of sodium fluoride.

Method: In the first experiment aluminum fluoride, AlF_3, was given in the drinking water, for 45 weeks, to three groups of rats in concentrations of 0.5 ppm, 5 ppm and 50 ppm (as Al^{3+}) starting at about 4 months of age. A fourth control group received only distilled water for drinking. In the second experiment, in addition to having a control group, two groups of rats received 0.5 ppm aluminum fluoride or 2.1 ppm of sodium fluoride. Both the aluminum fluoride and sodium fluoride gave a fluoride level of about 1 ppm. Aluminum fluoride was of special interest due to its lipid solubility and ability to pass through hydrophobic membranes. After sacrifice, the brain aluminum content was measured and histological examination made including study of the cortex, hippocampus and brain blood vessels. Tissue staining was done by the Morin fluorescent stain method, for aluminum, hematoxylin and eosin staining, cresyl-violet staining and by using the Bielchowsky silver stain. An immunohistochemical method was used to test for the immunoglobulin IgM. Vascular inclusions were studied by X-ray pulsation diffraction patterns and scanning electronmicroscopy. The kidneys were examined including histological examination and measurement of aluminum content.

Results:

In the first experiment a high mortality rate occurred in the animals receiving the lowest dose of aluminum fluoride: 0.5 ppm with 1 ppm of fluoride. Eighty percent of this group died before the end of the experiment at 45 weeks. Only a few animals died in other groups and the increased mortality in the 0.5 ppm aluminum fluoride group was significant. They also looked poorly prior to their deaths with thinner hair. Their exposed skin was bronzed, mottled or flaky. Their teeth and toe nails were excessively dark. The same high mortality in this group also occurred in the second experiment. When autopsies were performed on the bodies of the animals in the second experiment, a number of disease-related changes were found in the animals receiving aluminum fluoride or sodium fluoride including splenomegaly and *mycoplasma pulmonis* lung infections. The respiratory infection, which is common in rodents, was always much more virulent in the animals receiving the AlF_3 or the NaF. There appeared to be a general impairment

in the immune capacities of the treated animals. The animals treated with aluminum fluoride were most affected.

In both experiments deaths occurred in the 0.5 ppm AlF_3 dose groups when the additional stress of a behavioral training regime was imposed. It was as if an already diminished immune capacity was unable to tolerate the stress induced by the behavioral manipulations.

In the first experiment, all the AlF_3-exposed groups had increased brain levels of aluminum. These were about double those found in the control animals. No dose-response relationship occurred. Despite a 100-fold difference in dose all three groups had the same level of aluminum in the brain.

In the second experiment it was found that the prolonged administration of low levels of sodium fluoride, 2.1 ppm, corresponding to 1 ppm of fluoride, also resulted in an elevated brain level of aluminum. The aluminum was considered to have originated in the food provided to the animals. In the first experiment, for the animals receiving AlF_3, histologically, there was a reduction in the number of cells in the hippocampus in areas CA1 and CA3 together with a disorganization of the hippocampal pyramidal cells. Changes were observed in both the phosphorylated and nonphosphorylated neurofilaments in the neocortex which are usually considered to be related to cell dysfunction. Aluminum was found in brain blood vessels frequently in large amounts. It was particularly prominent in areas where blood vessels were most dense such as the median eminence. It was largely confined to the interior of blood vessels both large and small. The obvious presence of these inclusions in the blood vessels was seen to cause reductions in cerebral blood flow and most certainly a reduction in aerobic metabolism.

In the second study the same Al-positive inclusions were found in the group receiving 0.5 ppm of AlF_3. A few of these were also found in the group receiving 2.1 ppm sodium fluoride together with other nonaluminum types of inclusions that reacted positively for the immunoglobulin IgM. The inclusions were seen have the effect of reducing the amount of blood which could flow through the vessels together with producing a turbulence in blood flow. The turbulence was considered likely to reduce the transport of oxygen, nutrients, and waste products across the vessel walls.

On scanning electronmicroscopy, the vascular inclusions, which were presumed from the staining to contain aluminum, had a rather spiky crystalline configuration resembling an inorganic crystal. The presumed IgM inclusions had a more complex shape typical of an organic crystal. On X-ray diffraction spectrum examination, the presumed Al inclusions showed a peak corresponding to aluminum. For the presumed IgM inclusions the diffraction spectrum showed a peak corresponding to a high level of sulfur. This was seen to be consistent with the paired sulfur bonds in the immunoglobulins. These analyses were seen to confirm what had been assumed on the basis of histological and immunohistologic procedures.

In both studies the low dose of AlF_3 produced the highest mortality rates, the greatest signs of poor health, and substantial neural aberrations. Higher doses produced fewer deaths, healthier animals, and few neurological faults. Since all the

doses of the AlF_3 produced the same levels of Al in the brains, it was wondered if the difference in toxicity might be related to the difference in the amount of fluoride in the different dose levels.

In the second study, when rats were given the same amount of fluoride (2.1 ppm of sodium fluoride or 1 ppm of fluoride) in the form of sodium fluoride as was in the low dose of AlF_3 (0.5 ppm ionic aluminum with 1 ppm of fluoride) in the first study, no increase in mortality was found. The data were considered to indicate that as the doses of AlF_3 increased, the increasing amounts of fluoride must have acted to offset the devastating effects of the aluminum. It was presumed that a protective level of fluoride was achieved at the 5 and 50 ppm Al dose levels.

In the second study, the AlF_3-treated animals had more cortical cell loss and more cells with clear-cut structural anomalies than did the NaF-treated animals. The AlF_3-treated animals had cortical aberrancies in the upper layers, 2 and 3, and in the lower layers, 5 and 6. Cells in layer 4 were unaffected in all groups.

Neuronal abnormalities were observed in the sodium fluoride treated animals, however, especially in the deeper cell layers. Hemispheric differences were found in both the AlF_3 and NaF groups which were consistent in the groups. Cell losses were found in the hippocampal CA3 area and in the molecular layers of the dentate gyrus in the AlF_3-treated animals who also had plentiful deformations of the neurones of the CA1 and CA4 areas.

Both the AlF- and NaF-treated animals had substantial numbers of argentophilic cells on Bielchowsky staining and these cells showed condensed Nissl substance with hematoxylin and eosin staining. The NaF treatment also produced distortions of cells and, in some rats, cell losses could be demonstrated in particular brain regions. Both AlF_3 and NaF induced vascular inclusions, although of a different character: the AlF_3 produced the Al-based particles and the NaF produced the IgM inclusions. AlF_3 and NaF were seen to produce different reactions within the vascular tissue and the results of the first experiment were interpreted as being an interaction between aluminum and fluoride.

Pathological changes were found in the kidneys of animals in both the AlF_3 and NaF groups. Aluminum-containing deposits were found in the kidney blood vessels, and the renal aluminum content was doubled when the rats drank the AlF_3 water. The kidneys from rats drinking the NaF-treated water exhibited glomerular hypercellularity and mesangial proliferation together with patchy focal nephritis.

Discussion and Conclusions:

It was unclear why the greatest impairments and mortality rates were found in the animals with the lowest level of added AlF_3, 0.5 ppm as Al^{3+}. Why higher levels of AlF_3 produced less impairment, fewer deaths and generally healthier animals than the low levels remained a mystery. It is possible, based primarily on epidemiological reports, that fluoride, at certain low levels, may exert a protective effect against the aluminum when given at a certain absolute level. The general aluminum levels of brain tissue was the same in the treated groups, in both experiments, at a level about double that of the control groups. What differed in the higher AlF_3 groups was the amount of fluoride in the complexes. If the amount in

the lowest level groups was insufficient to provide protection but above a protection threshold in the higher dose groups, then this could account for the findings.

The immune deficiencies found in the animals support the general thesis of dementia as a microvascular-immune disease beginning with attacks on the integrity of the capillary linings in the brain and other organs. This could be due to the mechanisms involved in the formation of the inclusions found in the vessels. Damage to the vessel linings could provide the locations for the deposits of aluminum-containing materials, immunoglobulin, cholesterol, albumin, collagen, or amyloid. The brain's metabolic activity would be reduced hand in hand with the initiation of the innate immune response to foreign matter and the subsequent cascade of immunological responses. The combination of reduced aerobic metabolism and the immune reactions would act to enhance each other with the result being a progressive disease. A number of possible protective measures were seen to be suggested by the data:

1. Protecting the blood vessel linings from attack. If free radicals were involved prevention might be possible with scavengers, vitamin E and drugs like allopurinol.

2. Protection of the immune system from toxins and events that tend to reduce its efficiency. More attention might be paid towards discovering the interactions between the environmental factors and overall immune competencies including the effects of aluminum and other reactive metals.

3. Reducing any further impediments to blood flow. Since the effect of AlF_3 is largely to reduce the effectiveness of transport of oxygen and nutrients across the capillary walls, as well as the efficient removal of waste materials, every effort should be made to reduce any further impediment to blood flow. Treatments that reduce hypertension which impairs blood flow would be helpful especially those active at the blood vessels themselves.

4. Maintaining an effective cardiovascular system. Exercise and overall fitness might be encouraged.

5. Understanding the reciprocal relationship between the use of nonsteroidal anti-inflammatory drugs, other than aspirin, and dementia. Medication may have a role in more than just the early phase of the disease-related immune response in the brain.

6. Understanding the role of fluoride in providing protection from certain types of toxins. Based on knowledge of this element's own toxicity, it cannot be used without understanding the risks it imposes at various doses to different organs. The possible benefits from its use must be balanced with its potential harm. Nevertheless, there is evidence supporting a neuroprotective effect of fluoride in water supplies in regard to dementia from epidemiological studies whether or not aluminum is also in the water. If the protective mechanisms are understood, it may be possible to find other agents to provide them with less harmful side effects.

Key words: Aluminum-fluoride; Amyloid; Brain; Cerebrovasculature; Hippocampus; Neurotoxicity; Rat; Sodium fluoride.

Reprints: Robert L Isaacson, Department of Psychology, Binghamton University, Binghamton, New York 13902-6000, USA. Fax: +1-607-777-4890.
E-mail: isaacson@binghamton.edu

FLUORIDE SALTS ARE NO BETTER AT PREVENTING NEW VERTEBRAL FRACTURES THAN CALCIUM-VITAMIN D IN POSTMENOPAUSAL OSTEOPOROSIS - THE FAVOS STUDY

P J Meunier, J L Sebert, J Y Reginster, D Briancon, T Appelboom, P Netter, G Loeb, A Rouillon, S Barry, J C Evreux, B Avouac and X Marchandise
Lyon, France

Abstract from *Osteoporosis International 8* (1) 4-12 1998

Although fluoride salts have been shown to be capable of linearly increasing spinal bone mineral density (BMD) in postmenopausal osteoporosis, the effects of this gain in density on the vertebral fracture rate remain controversial. We conducted a 2-year multicenter, prospective, randomized, double-masked clinical trial in 354 osteoporotic women with vertebral fractures (mean age 65.7 years). They received either fluoride (208 patients): given as sodium fluoride (50 mg/day) or as monofluorophosphate (200 mg/day or 150 mg/day), or a placebo (146 patients), All patients received daily supplements of 1 g of calcium (Ca) and 800 IU of vitamin D-2 (D). A 1-year open follow-up on Ca-D was obtained in 124 patients. After 2 years the fluoride group and the Ca-D group had increased their lumbar BMD by 10.8% and 2.4% respectively ($p = 0.0001$). However, the rate of patients with at least one new vertebral fracture, defined by semiquantitative assessment and evaluable on an intention-to-treat basis in 89% of patients, was similar in the fluoride groups and the Ca-D group. No difference between the three fluoride regimens was found. The percentage of patients with nonvertebral fractures was not different in the fluoride and Ca-D groups (1.9% and 1.4% respectively for hip fractures). A lower limb pain syndrome occurred more frequently in the fluoride groups. In the 124 patients followed for 1 year after cessation of fluoride therapy, the percentage of patients with at least one new vertebral fracture after 36 months was identical to the percentages in the previous fluoride group and the Ca-D group. We conclude that fluoride-Ca-D regimen was no more effective that Ca-D supplements for the prevention of new vertebral fractures in women with postmenopausal osteoporosis.

Key words: Calcium; Monofluorophosphate; Postmenopausal osteoporosis; Sodium fluoride; Vertebral fracture; Vitamin D.

Reprints: P J Meunier, Hopital Edouard Herriot, Department of Rheumatology and Bone Diseases, Pavillon F, F-69437 Lyon 3, France.

SYSTEMIC EFFECTS OF FLUORIDATED WATER ON RATS

E M A Boeckhhaebisch and R M Oliveira
São Paulo, Brazil

Abstract from *Arquivos de Biologia e Tecnologia 40* (1) 57-68 1997

The biological effects of fluoride were investigated on rats fed pure spring water (natural F^- concentration = 0.2 ppm) or spring water enriched with NaF to result 0.8, 1.1 or 2.2 ppm F^- during 180 days. The main effects of fluoride have been: 1) Electrocardiographic recordings showed a significant reduction of the electrical systole (QTc). 2) Body weight gain was unaffected by the treatment. 3) Bivalent cations (Ca^{2+}, Mg^{2+} and Zn^{2+}) were importantly reduced in adrenals,

electrical systole (QTc). 2) Body weight gain was unaffected by the treatment. 3) Bivalent cations (Ca^{2+}, Mg^{2+} and Zn^{2+}) were importantly reduced in adrenals, ventricular myocardium, and bone. 4) Na+ concentration was strikingly increased in aorta, lung and joint of treated animals. 5) In testis, we observed Ca^{2+} and Na^{+} retention. 6) Zinc ions were depleted in most tissues, except kidney and submandibular salivary gland. Our results revealed that chronic administration of fluoridated water does influence systemic biochemical homeostasis in rats, without evoking any overt sign of fluorosis. Moreover, the definition of a "safe" concentration of F^{-} in public water must consider that the dose at which beneficial effects as caries reduction are attained is not far away from that one which causes chronic yet subclinical toxic effects.

Key words: Biological effects; Electrolyte metabolism; Fluoridated drinking water; Rats.
Reprints: E M A Boeckhhaebisch, University of Sao Paulo Institute of Biomedical Science, Department of Pharmacology, Cidade University, Br-05508900 Sao Paulo, Brazil.

RISK FACTORS ASSOCIATED WITH FLUOROSIS IN A NON-FLUORIDATED POPULATION IN NORWAY

N J Wang, A M Gropen and B Ogaard
Oslo, Norway

Abstract from *Community Dentistry and Oral Epidemiology 25* (6) 396-401 1997

In Norway there is no water fluoridation and little naturally occurring fluoride in drinking water. Fluoride toothpaste is used by 95% of the population and there is a long tradition of fluoride supplement use. The purpose of this study was to record the prevalence and severity of dental fluorosis in 8-year-old children and relate this to systemic fluoride exposure (supplements and toothpaste). All children (n=551, born 1988) in a municipality in Norway were invited to participate. Dental fluorosis on the buccal surface of the upper permanent incisors was recorded according to the Thylstrup-Fejerskov index (TF). Parents provided data on use of supplements and toothpaste. Complete data were obtained from 383 children. Sixty-seven percent of the children had used fluoride supplements regularly during childhood. At 8 months or earlier, the teeth of 26% of the children, and at age 14 months or earlier the teeth of 82%, were being brushed. Among children who used fluoride supplements regularly, periodically, seldom and not at all, 45%, 21%, 10% and 0%, respectively, had dental fluorosis. The dental fluorosis was mild (TF=1) in 87% of the cases. Bivariate and multivariate analyses showed that, in addition to use of fluoride supplements, starting toothbrushing at an early age was associated with higher prevalence of dental fluorosis. The child's birth weight and liking for or swallowing of toothpaste did not influence the prevalence of fluorosis. Risk factors for fluorosis were use of toothpaste before the age of 14 months and regular use of fluoride supplements during childhood.

Key words: Dental fluorosis; Epidemiology; Fluoride supplements; Fluoride toothpaste; TF index.
Reprints: N J Wang, University of Oslo, Department of Pedodontics and Caries Prophylaxis, Box 1109, Blindern, N-0317 Oslo, Norway.

THE PERILS OF PROGRESS

The Health and Environment Hazards of Modern Technology, & What You Can Do About Them

by John Ashton and Ron Laura

(University of New South Wales Press, University of New South Wales, Sydney 2052, Australia. 346 pages with figures. Recommended retail price A$29.95.)

Reviewed by Bill Wilson

The foreword, by Emeritus Professor Charles Birch of the University of Sydney and Challis professor of biology, states that this is a book whose time had come and indeed was overdue. Dr John Ashton, a chemist, and Dr Ron Laura, a Professor of Education, have written a comprehensive, thought-provoking book with 52 pages of references and an excellent index.

Part one of the book briefly outlines technological progress, from the Industrial Revolution to the present day, with the increase in scientific knowledge. The extent to which the consequences of the use of technology, in electricity and electronics, food and water processing, imperil human health and affect the environment is discussed in more detail in Parts two to five.

The possible dangers of electromagnetic fields and radiation from power and communications lines, computers, VDUs and microwave ovens are analysed in Part two. The authors conclude that there is no certainty of safety in the use of these power sources.

Food and water technology impact on humans is covered in Parts three and four. Reduction in nutritional value of processed food and the possible toxicity of food additives, which occur due to inadequate health regulations, are discussed. Chlorine added to water and aluminium from water, food additives and dissolved from cooking vessels indicate cause for concern. A whole chapter discusses the water fluoridation debate, fully examines the evidence of benefit and risks, and concludes: "Whether the fluoridation campaign must be indicted as a public health campaign gone badly wrong is a judgement best reserved for the reader. Whatever the judgement, it is incontestable that the prevention of tooth decay is not the bottom-line of the fluoridation debate, if the panacea has become the poison."

Part five discusses air conditioning and artificial light in relation to "sick building syndrome" and the effect on health. The influence of excessive sound is also canvassed.

Each chapter ends with suggestions of what the reader can do to minimise the effects discussed in that chapter. Some of these suggestions are impractical for many people. This is the only criticism of a book which I strongly commend to both professionals and laypersons.

WHY I CHANGED MY MIND ABOUT WATER FLUORIDATION

John Colquhoun*

Former Advocate

To explain how I came to change my opinion about water fluoridation, I must go back to when I was an ardent advocate of the procedure. I now realize that I had learned, in my training in dentistry, only one side of the scientific controversy over fluoridation. I had been taught, and believed, that there was really no scientific case against fluoridation, and that only misinformed lay people and a few crackpot professionals were foolish enough to oppose it. I recall how, after I had been elected to a local government in Auckland (New Zealand's largest city, where I practised dentistry for many years and where I eventually became the Principal Dental Officer) I had fiercely – and, I now regret, rather arrogantly – poured scorn on another Council member (a lay person who had heard and accepted the case against fluoridation) and persuaded the Mayor and majority of my fellow councillors to agree to fluoridation of our water supply.

A few years later, when I had become the city's Principal Dental Officer, I published a paper in the *New Zealand Dental Journal* that reported how children's tooth decay had declined in the city following fluoridation of its water, to which I attributed the decline, pointing out that the greatest benefit appeared to be in low-income areas [1]. My duties as a public servant included supervision of the city's school dental clinics, which were part of a national School Dental Service which provided regular six-monthly dental treatment, with strictly enforced uniform diagnostic standards, to almost all (98 percent) school children up to the age of 12 or 13 years. I thus had access to treatment records, and therefore tooth decay rates, of virtually all the city's children. In the study I claimed that such treatment statistics "provide a valid measure of the dental health of our child population" [1]. That claim was accepted by my professional colleagues, and the study is cited in the official history of the New Zealand Dental Association [2].

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* School of Education, University of Auckland, Private Bag 92019, Auckland, New Zealand.

First published in *Perspectives in Biology and Medicine 41* 29-44 1997. Reprinted with permission in *Fluoride*, Journal of the International Society for Fluoride Research.

INFORMATION CONFIDED

I was so articulate and successful in my support of water fluoridation that my public service superiors in our capital city, Wellington, approached me and asked me to make fluoridation the subject of a world study tour in 1980 – after which I would become their expert on fluoridation and lead a campaign to promote fluoridation in those parts of New Zealand which had resisted having fluoride put into their drinking water.

Before I left on the tour my superiors confided to me that they were worried about some new evidence which had become available: information they had collected on the amount of treatment children were receiving in our school dental clinics seemed to show that tooth decay was declining just as much in places in New Zealand where fluoride had not been added to the water supply. But they felt sure that, when they had collected more detailed information, on *all* children (especially the oldest treated, 12-13 year age group) from *all* fluoridated and *all* nonfluoridated places [3] – information which they would start to collect while was I away on my tour – it would reveal that the teeth were better in the fluoridated places: not the 50 to 60 percent difference which we had always claimed resulted from fluoridation, but a significant difference nonetheless. They thought that the decline in tooth decay in the nonfluoridated places must have resulted from the use of fluoride toothpastes and fluoride supplements, and from fluoride applications to the children's teeth in dental clinics, which we had started at the same time as fluoridation. Being a keen fluoridationist, I readily accepted their explanation. Previously, of course, we had assured the public that the only really effective way to reduce tooth decay was to add fluoride to the water supply.

WORLD STUDY TOUR

My world study tour took me to North America, Britain, Europe, Asia, and Australia [4]. In the United States I discussed fluoridation with Ernest Newbrun in San Francisco, Brian Burt in Ann Arbor, dental scientists and officials like John Small in Bethesda near Washington, DC, and others at the Centers for Disease Control in Atlanta. I then proceeded to Britain, where I met Michael Lennon, John Beale, Andrew Rugg-Gunn, and Neil Jenkins, as well as many other scientists and public health officials in Britain and Europe. Although I visited only pro-fluoridation research centers and scientists, I came across the same situation which concerned my superiors in New Zealand. Tooth decay was declining without water fluoridation. Again I was assured, however, that more extensive and thorough surveys would show

that fluoridation was the most effective and efficient way to reduce tooth decay. Such large-scale surveys, on very large numbers of children, were nearing completion in the United States, and the authorities conducting them promised to send me the results.

LESSON FROM HISTORY

I now realize that what my colleagues and I were doing was what the history of science shows all professionals do when their pet theory is confronted by disconcerting new evidence: they bend over backwards to explain away the new evidence. They try very hard to keep their theory intact – especially so if their own professional reputations depend on maintaining that theory. (Some time after I graduated in dentistry almost half a century ago, I also graduated in history studies, my special interest being the history of science – which may partly explain my re-examination of the fluoridation theory ahead of many of my fellow dentists.)

So I returned from my study tour reinforced in my pro-fluoridation beliefs by these reassurances from fluoridationists around the world. I expounded these beliefs to my superiors, and was duly appointed chairman of a national "Fluoridation Promotion Committee." I was instructed to inform the public, and my fellow professionals, that water fluoridation resulted in better children's teeth, when compared with places with no fluoridation.

Surprise: Teeth Better Without Fluoridation?

Before complying, I looked at the new dental statistics that had been collected while I was away for my own Health District, Auckland. These were for all children attending school dental clinics – virtually the entire child population of Auckland. To my surprise, they showed that fewer fillings had been required in the nonfluoridated part of my district than in the fluoridated part. When I obtained the same statistics from the districts to the north and south of mine – that is, from "Greater Auckland," which contains a quarter of New Zealand's population – the picture was the same: tooth decay had declined, but there was virtually no difference in tooth decay rates between the fluoridated and non fluoridated places. In fact, teeth were slightly better in the nonfluoridated areas. I wondered why I had not been sent the statistics for the rest of New Zealand. When I requested them, they were sent to me with a warning that they were not to be made public. Those for 1981 showed

that in most Health Districts the percentage of 12- and 13-year-old children who were free of tooth decay - that is, had perfect teeth - was greater in the non-fluoridated part of the district. Eventually the information was published [4].

Over the next few years these treatment statistics, collected for all children, showed that, when similar fluoridated and non-fluoridated areas were compared, child dental health continued to be slightly better in the non-fluoridated areas [5,6]. My professional colleagues, still strongly defensive of fluoridation, now claimed that treatment statistics did not provide a valid measure of child dental health, thus reversing their previous acceptance of such a measure when it had appeared to support fluoridation.

I did not carry out the instruction to tell people that teeth were better in the fluoridated areas. Instead, I wrote to my American colleagues and asked them for the results of the large-scale surveys they had carried out there. I did not receive an answer. Some years later, Dr John Yiamouyiannis obtained the results by then collected by resorting to the U.S. Freedom of Information Act, which compelled the authorities to release them. The surveys showed that there is little or no differences in tooth decay rates between fluoridated and nonfluoridated places throughout America [7]. Another publication using the same database, apparently intended to counter that finding, reported that when a more precise measurement of decay was used, a small benefit from fluoridation was shown (20 percent fewer decayed tooth surfaces, which is really less than one cavity per child) [8]. Serious errors in that report, acknowledged but not corrected, have been pointed out, including a lack of statistical analysis and a failure to report the percentages of decay-free children in the fluoridated and nonfluoridated areas [7].

Other large-scale surveys from United States, from Missouri and Arizona, have since revealed the same picture: no real benefit to teeth from fluoride in drinking water [9, 10]. For example, Professor Steelink in Tucson, AZ, obtained information on the dental status of all schoolchildren – 26,000 of them – as well as information on the fluoride content of Tucson water [10]. He found: "When we plotted the incidence of tooth decay versus fluoride content in a child's neighborhood drinking water, a positive correlation was revealed. In other words, the more fluoride a child drank, the more cavities appeared in the teeth" [11].

From other lands – Australia, Britain, Canada, Sri Lanka, Greece, Malta, Spain, Hungary, and India – a similar situation has been revealed: either little or no relation between water fluoride and tooth decay, or a positive one (more fluoride, more decay) [12-17]. For example, over 30 years Professor Teotia and his team in India have examined the teeth of some 400,000 children. They found that tooth decay increases as fluoride intake increases. Tooth decay, they decided, results from a deficiency of calcium and an excess of fluoride [17].

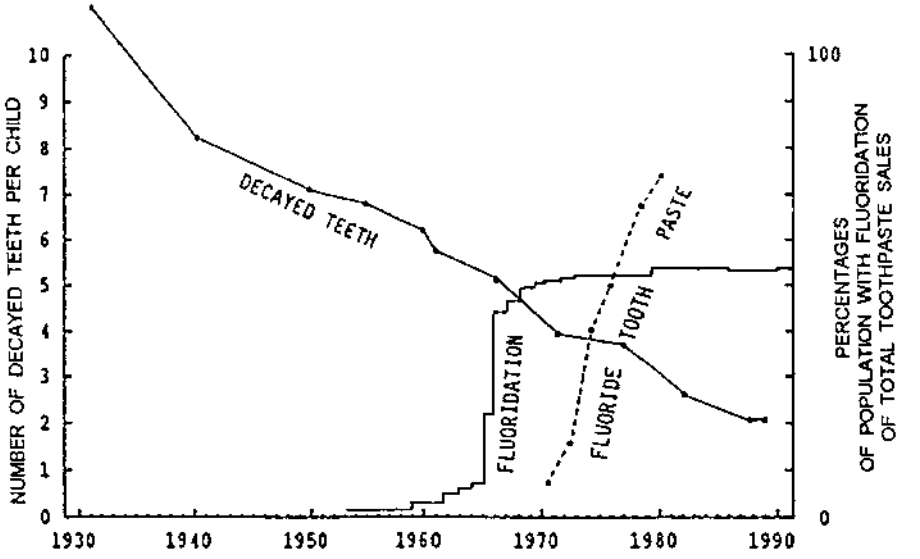

FIGURE 1. — 50-year decline in tooth decay of 5-year-olds.
SOURCE. — Compiled from Health Department records of 5-year-olds' tooth decay 1930-1990, fluoridation, and fluoride toothpaste sales.

CAUSE OF DECLINE IN TOOTH DECAY

At first I thought, with my colleagues, that other uses of fluoride must have been the main cause of the decline in tooth decay throughout the western world. But what came to worry me about that argument was the fact that, in the nonfluoridated part of my city, where decay had also declined dramatically, very few children used fluoride toothpaste, many had not received fluoride applications to their teeth, and hardly any had been given fluoride tablets. So I obtained the national figures on tooth decay rates of five-year-olds from our dental clinics which had served large numbers of these children from the 1930s on [18]. They show that tooth decay had started to decline well before we had started to use fluorides (Fig. 1). Also, the decline has continued after all children had received fluoride all their lives, so the continuing decline could not be because of fluoride. The fewer figures available for older children are consistent with the above pattern of decline [18]. So fluorides, while possibly contributing, could not be the main cause of the reduction in tooth decay.

So what did cause this decline, which we find in most industrialized countries? I do not know the answer for sure, but we do know that after the second world war there was a rise in the standard of living of many people. In my country there has been a tremendous increase in the consumption of

fresh fruit and vegetables since the 1930s, assisted by the introduction of household refrigerators [19]. There has also been an eightfold increase in the consumption per head of cheese, which we now know has anti-decay properties [19, 20]. These nutritional changes, accompanied by a continuing decline in tooth decay, started before the introduction of fluorides.

The influence of general nutrition in protection against tooth decay has been well described in the past [21], but is largely ignored by the fluoride enthusiasts, who insist that fluorides have been the main contributor to improved dental health. The increase in tooth decay in third-world countries, much of which has been attributed to worsening nutrition [22], lends support to the argument that improved nutrition in developed countries contributed to improved dental health.

Flawed Studies

The studies showing little if any benefit from fluoridation have been published since 1980. Are there contrary findings? Yes: many more studies, published in dental professional journals, claim that there is a benefit to teeth from water fluoride. An example is a recent study from New Zealand [23], carried out in the southernmost area of the country [23]. Throughout New Zealand there is a range of tooth decay rates, from very high to very low, occurring in both fluoridated and nonfluoridated areas. The same situation exists in other countries.

What the pro-fluoride academics at our dental school did was to select from that southern area four communities: one nonfluoridated, two fluoridated, and another which had stopped fluoridation a few years earlier. Although information on decay rates in all these areas was available to them, from the school dental service, they chose for their study the one nonfluoridated community with the highest decay rate and two fluoridated ones with low decay rates, and compared these with the recently stopped fluoridated one, which happened to have medium decay rates (both before and after it had stopped fluoridation). The teeth of randomly selected samples of children from each community were examined. The chosen communities, of course, had not been randomly selected. The results, first published with much publicity in the news media, showed over 50 percent less tooth decay in the fluoridated communities, with the recently defluoridated town in a "middle" position (see left side of Fig. 2). When I obtained the decay rates for *all* children in *all* the fluoridated and *all* the nonfluoridated areas in that part of New Zealand, as well as the decay rates for *all* children in the recently defluoridated town, they revealed that there are virtually no differences in tooth decay rates related to fluoridation (see right side of Fig. 2).

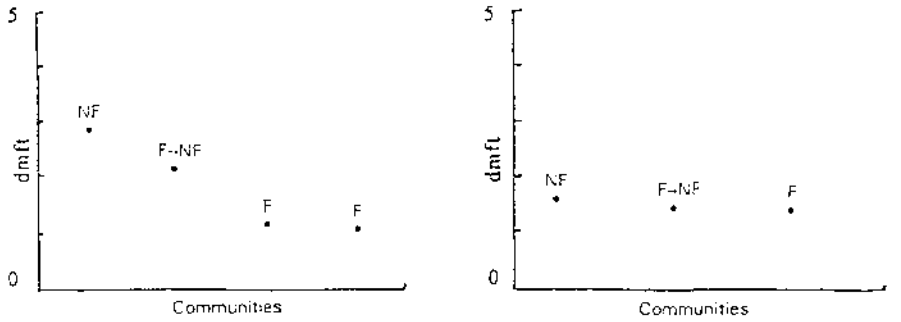

FIGURE 2. – Left: results of South Island dental survey of samples of 5-year-olds from selected areas. See [23]. Right: Results for all 5-year-olds in all nonfluoridated, fluoridated, and defluoridated areas. (School Dental Service records).

When I confronted the authors with this information, they retorted that the results of their study were consistent with other studies. And of course it is true that many similar studies have been published in the dental professional literature. It is easy to see how the consistent results are obtained: an appropriate selection of the communities being compared. There is another factor: most pro-fluoridation studies (including this New Zealand one) were not "blind" – that is, the examiners knew which children received fluoride and which did not. Diagnosis of tooth decay is a very subjective exercise, and most of the examiners were keen fluoridationists, so it is easy to see how their bias could affect their results. It is just not possible to find a blind fluoridation study in which the fluoridated and nonfluoridated popultions were similar and chosen randomly.

EARLY FLAWED STUDIES

One of the early fluoridation studies listed in the textbooks is a New Zealand one, the "Hastings Fluoridation Experiment" (the term "experiment" was later dropped because the locals objected to being experimented on) [24]. I obtained the Health Department's fluoridation files under my own country's "Official Information" legislation. They revealed how a fluoridation trial can, in effect, be rigged [25]. The school dentists in the area of the experiment were instructed to change their method of diagnosing tooth decay, so that they recorded much less decay after fluoridation began. Before the experiment they had filled (and classified as "decayed") teeth with any small catch on the surface, before it had penetrated the outer enamel layer. After the experiment began, they filled (and classified as "decayed") only teeth with cavities which penetrated the outer enamel layer. It is easy to see why a sudden drop in the numbers of "decayed and filled" teeth occurred. This change in method of diagnosis was not reported in any of the published accounts of the experiment.

Another city, Napier, which was not fluoridated but had otherwise identical drinking water, was at first included in the experiment as an "ideal control" – to show how tooth decay did not decline the same as in fluoridated Hastings. But when tooth decay actually declined more in the nonfluoridated control city than in the fluoridated one, in spite of the instructions to find fewer cavities in the fluoridated one, the control was dropped and the experiment proceeded with no control. (The claimed excuse was that a previously unknown trace element, molybdenum, had been discovered in some of the soil of the control city, making tooth decay levels there unusually low [26], but this excuse is not supported by available information, from the files or elsewhere, on decay levels throughout New Zealand).

The initial sudden decline in tooth decay in the fluoridated city, plus the continuing decline which we now know was occurring everywhere else in New Zealand, were claimed to prove the success of fluoridation. These revelations from government files were published in the international environmental journal, *The Ecologist*, and presented in 1987 at the 56th Congress of the Australian and New Zealand Association for the Advancement of Science [27].

When I re-examined the classic fluoridation studies, which had been presented to me in the text books during my training, I found, as others had before me, that they also contained serious flaws [28-30]. The earliest set, which purported to show an inverse relationship between tooth decay prevalence and naturally occurring water fluoride concentrations, are flawed mainly by their nonrandom methods of selecting data. The later set, the "fluoridation trials" at Newburgh, Grand Rapids, Evanston, and Brantford, display inadequate baselines, negligible statistical analysis, and especially a failure to recognize large variations in tooth decay prevalence in the control communities. We really cannot know whether or not some of the tooth decay reductions reported in those early studies were due to water fluoride.

I do not believe that the selection and bias that apparently occurred was necessarily deliberate. Enthusiasts for a theory can fool themselves very often, and persuade themselves and others that their activities are genuinely scientific. I am also aware that, after 50 years of widespread acceptance and endorsement of fluoridation, many scholars (including the reviewers of this essay) may find it difficult to accept the claim that the original fluoridation studies were invalid. That is why some of us, who have reached that conclusion, have submitted an invitation to examine and discuss new and old evidence "in the hope that at least some kind of scholarly debate will ensue" [31].

However, whether or not the early studies were valid, new evidence strongly indicates that water fluoridation today is of little if any value. Moreover, it is now widely conceded that the main action of fluoride on teeth is a topical one (at the surface of the teeth), not a systemic one as previously thought, so that there is negligible benefit from swallowing fluoride [32].

Harm from Fluoridation

The other kind of evidence which changed my mind was that of *harm* from fluoridation. We had always assured the public that there was absolutely no possibility of any harm. We admitted that a small percentage of children would have a slight mottling of their teeth, caused by the fluoride, but this disturbance in the formation of tooth enamel would, we asserted, be very mild and was nothing to worry about. It was, we asserted, not really a sign of toxicity (which was how the early literature on clinical effects of fluoride had described it) but was only at most a slight, purely cosmetic change, and no threat to health. In fact, we claimed that only an expert could ever detect it.

HARM TO TEETH

So it came as a shock to me when I discovered that in my own fluoridated city some children had teeth like those in Fig. 3. This kind of mottling answered the description of dental fluorosis (bilateral diffuse opacities along the growth lines of the enamel). Some of the children with these teeth had used fluoride toothpaste and swallowed much of it. But I could not find children with this kind of fluorosis in the nonfluoridated parts of my Health District, except in children who had been given fluoride tablets at the recommended dose of that time.

I published my findings: 25 percent of children had dental fluorosis in fluoridated Auckland and around 3 percent had the severer (discolored or pitted) degree of the condition [33]. At first the authorities vigorously denied that fluoride was causing this unsightly mottling. However, the following year another Auckland study, intended to discount my finding, reported almost identical prevalences and severity, and recommended lowering the water fluoride level to below 1 ppm [34]. Others in New Zealand and the United States have reported similar findings. All these studies were reviewed in the journal of the International Society for Fluoride Research [35]. The same unhappy result of systemic administration of fluoride has been reported in children who received fluoride supplements [36]. As a result, in New Zealand as elsewhere, the doses of fluoride tablets were drastically reduced, and parents were warned to reduce the amount of fluoride toothpaste used by their children, and to caution them not swallow any. Fluoridationists would not at first admit that fluoridated water contributed to the unsightly mottling – though later, in some countries including New Zealand, they also recommended lowering the level of fluoride in the water. They still insist that the benefit to teeth outweighs any harm.

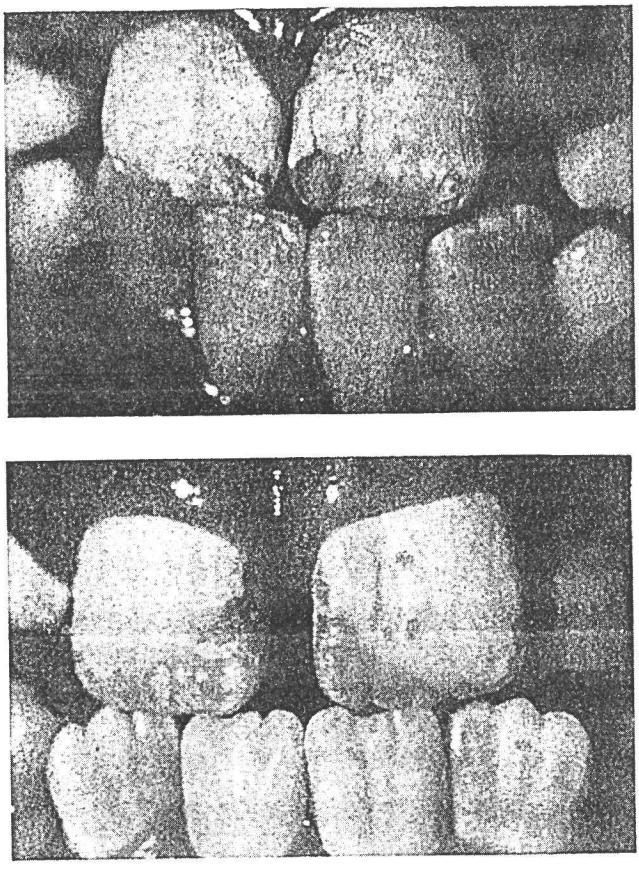

Figure 3. – Examples of dental fluorosis in 8- and 9-year old children who grew up in fluoridated Auckland, New Zealand.

WEAKENED BONES

Common sense should tell us that if a poison circulating in a child's body can damage the tooth-forming cells, then other harm also is likely. We had always admitted that fluoride in excess can damage bones, as well as teeth.

By 1983 I was thoroughly convinced that fluoridation caused more harm than good. I expressed the opinion that some of these children with dental fluorosis could, just possibly, have also suffered harm to their bones [Letter to Auckland Regional Authority, January 1984]. This opinion brought scorn and derision: there was absolutely no evidence, my dental colleagues asserted, of any other harm from low levels of fluoride intake, other than mottling of the teeth.

Six years later, the first study reporting an association between fluoridated water and hip fractures in the elderly was published [37]. It was a large-scale one. Computerization has made possible the accumulation of vast data banks of information on various diseases. Hip fracture rates have increased dramatically, independently of the increasing age of populations. Seven other studies have now reported this association between low water fluoride levels and hip fractures [38-44]. Have there been contrary findings? Yes; but most of the studies claiming no association are of small numbers of cases, over short periods of time, which one would not expect to show any association [45, 46]. Another, comparing a fluoridated and a nonfluoridated Canadian community, also found an association in males but not in females, which hardly proves there is no difference in all cases [47]. Our fluoridationists claim that the studies which do show such an association are only epidemiological ones, not clinical ones, and so are not conclusive evidence.

But in addition to these epidemiological studies, clinical trials have demonstrated that when fluoride was used in an attempt to treat osteoporosis (in the belief it strengthened bones), it actually caused more hip fractures [48-52]. That is, when fluoride accumulates in bones, it weakens them. We have always known that only around half of any fluoride we swallow is excreted in our urine; the rest accumulates in our bones [53, 54]. But we believed that the accumulation would be insignificant at the low fluoride levels of fluoridated water. However, researchers in Finland during the 1980s reported that people who lived 10 years or more in that country's one fluoridated city, Kuopio, had accumulated extremely high levels of fluoride in their bones – thousands of parts per million – especially osteoporosis sufferers and people with impaired kidney function [55, 56]. After this research was published, Finland stopped fluoridation altogether. But that information has been ignored by our fluoridationists.

BONE CANCER?

An association with hip fracture is not the only evidence of harm to bones from fluoridation. Five years ago, animal experiments were reported of a fluoride-related incidence of a rare bone cancer, called osteosarcoma, in young male rats [57]. Why only the male animals got the bone cancer is not certain, but another study has reported that fluoride at very low levels can interfere with the male hormone, testosterone [58]. That hormone is involved in bone growth in males but not in females.

This finding was dismissed by fluoridation promoters as only "equivocal evidence," unlikely to be important for humans. But it has now been found that the same rare bone cancer has increased dramatically in young *human* males – teenage boys aged 9 to 19 – in the fluoridated areas of America but not in the nonfluoridated areas [59]. The New Jersey Department of Health reported

osteosarcoma rates were three to seven times higher in its fluoridated areas than in its nonfluoridated areas [60].

Once again, our fluoridationists are claiming that this evidence does not "conclusively" demonstrate that fluoride caused the cancers, and they cite small-scale studies indicating no association. One study claimed that fluoride might even be protective against osteosarcoma [61]; yet it included only 42 males in its 130 cases, which meant the cases were not typical of the disease, because osteosarcoma is routinely found to be more common in males. Also, the case-control method used was quite inappropriate, being based on an assumption that if ingested fluoride was the cause, osteosarcoma victims would require higher fluoride exposure than those without the disease. The possibility that such victims might be more susceptible to equal fluoride exposures was ignored. All these counter-claims have been subjected to critical scrutiny which suggests they are flawed [62, 63]. Nonetheless, the pro-fluoride lobbyists continue to insist that water fluoridation should continue because, in their view, the benefits to teeth outweigh the possibility of harm. Many dispute that assessment.

OTHER EVIDENCE OF HARM

There is much more evidence that tooth mottling is not the only harm caused by fluoridated water. Polish researchers, using a new computerized method of X-ray diagnosis, reported that boys with dental fluorosis also exhibit bone structure disturbances [64]. Even more chilling is the evidence from China that children with dental fluorosis have on average lower intelligence scores [65, 66]. This finding is supported by a recently published animal experiment in America, which showed that fluoride also accumulated in certain areas of the brain, affecting behavior and the ability to learn [67].

Endorsements Not Universal

Concerning the oft-repeated observation that fluoridation has enjoyed overwhelming scientific endorsement, one should remember that even strongly supported theories have eventually been revised or replaced. From the outset, distinguished and reputable scientists opposed fluoridation, in spite of considerable intimidation and pressure [68, 69].

Most of the world has rejected fluoridation. Only America where it originated, and countries under strong American influence persist in the practice. Denmark banned fluoridation when its National Agency for Environmental Protection, after consulting the widest possible range of scientific sources, pointed out that the long-term effects of low fluoride intakes on certain groups in the population (for example, persons with reduced kidney function), were insufficiently known [70]. Sweden also rejected fluoridation on the recommendation of a special Fluoride Commission, which included among

its reasons that: "The combined and long-term environmental effects of fluoride are insufficiently known" [71]. Holland banned fluoridation after a group of medical practitioners presented evidence that it caused reversible neuromuscular and gastrointestinal harm to some individuals in the population [72].

Environmental scientists, as well as many others, tend to doubt fluoridation. In the United States, scientists employed by the Environmental Protection Agency have publicly disavowed support for their employer's pro-fluoridation policies [73]. The orthodox medical establishment, rather weak or even ignorant on environmental issues, persist in their support, as do most dentists, who tend to be almost fanatical about the subject. In English-speaking countries, unfortunately, the medical profession and its allied pharmaceutical lobby (the people who sell fluoride) seem to have more political influence than environmentalists.

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The following **CRITICAL REVIEW**, by a professor at the School of Dentistry in San Francisco, has been sent to this journal. Dr Colquhoun's response is on pages 127-128.

WHY I CHANGED MY MIND ABOUT WATER FLUORIDATION by John Colquhoun
Perspectives in Biology and Medicine 41 (1) 29-44 Autumn 1997

Reviewed by Howard Pollick BDS MPH

I find this publication informational but biased in its details. I cannot conclude that water fluoridation is ineffective or harmful based on this paper, although Colquhoun attempts to persuade the reader of the correctness of his views. I base these conclusions on the following points.

1. In New Zealand there is a national School Dental Service that provided regular six-monthly dental treatment, with strictly enforced uniform diagnostic standards, to almost all (98 percent) school children up to the age of 12 or 13 years.

Over the next few years, these treatment statistics, collected for all children, showed that when similar fluoridated or unfluoridated areas were compared, child dental health continued to be slightly better in the fluoridated areas.

These are quotes from this paper which show that New Zealand has had a school-based dental treatment program and that, at least at one period of time Colquhoun acknowledges that fluoridation appeared to be of benefit. The school-based program provides treatment and prevention services, including application of fluorides and is a model of dental services that does not exist in many parts of the world, and not in California. It appears that Colquhoun has demonstrated that the school-based dental service, in part, has provided what fluoridation can provide. Colquhoun however does not address the issue of cost-effectiveness and presumably the dental service is far more expensive than fluoridation.

2. In comparing the work by Yiamouyiannis and that by Brunelle and Carlos of the U.S. 1986-87 national study on dental caries, Colquhoun states: 'Another publication using the same data base (Brunelle and Carlos) apparently intended to counter that findings (by Yiamouyiannis) reported that when a more precise measurement of decay was used, a small benefit from fluoridation was shown (20 percent fewer decayed tooth surfaces, which is really less than one cavity per child).' Since the publication by Yiamouyiannis includes an analysis of the publication by Brunelle and Carlos, then the journal Fluoride must have published Yiamouyiannis after the Journal of Dental Research published Brunelle and Carlos. Thus Colquhoun's conclusion that Brunelle and Carlos 'apparently intended to counter that findings (by Yiamouyiannis)' is misleading.

Colquhoun appears to have implied that because he didn't receive a reply from his American colleagues about the result of the national U.S. study and because Yiamouyiannis obtained the results 'resorting to the U.S. Freedom of Information Act' that the results would otherwise have not been published. On the contrary, they were published in 1989. (JA Brunelle. *Oral health of United States children, national and regional findings.* DHSS Publication No. (NIH) 89-2247, U.S. Government Printing Office, Washington, DC, 1989.)

Thus it appears that Colquhoun fuels the fires of an alleged conspiracy at the highest levels of dental research in the U.S. to withhold information from the public and to bias the findings.

3. Colquhoun refers to some authors by name without title and others by name with titles, with those whose work supports that of Colquhoun with their title, for example 'Dr John Yiamouyiannis', 'Professor Steelink' and 'Professor Teotia', as if by adding their title they are afforded a higher place in Colquhoun's hierarchy. This indicates bias.

4. The work of Teotia in India concerns areas with very high fluoride beyond the recommended concentrations for water fluoridation.

5. Colquhoun suggests that the improved nutrition (without regard to sugar consumption) in most industrialized countries that has occurred since the 1930s and after World War II may be more important than fluorides in the prevention of tooth decay. However, he acknowledges that 'I do not know the answer for sure', and indeed does not refer to any epidemiologic studies that have shown that general nutrition is a greater factor than fluorides in the prevention of tooth decay in communities where people do not generally suffer from malnutrition.

Malnutrition that accompanies wartime periods and remote and isolated communities and countries such as New Zealand in the 1930's has been linked with increases and decreases in tooth decay prevalence, depending on the availability of sugar. Colquhoun rightly points to 'The increase in tooth decay in third-world countries, much of which has been attributed to worsening nutrition.

6. Colquhoun points out 'the studies showing little if any benefit from fluoridation have been published since 1980. Are there contrary findings? Yes: many more studies, published in dental professional journals, claim that there is a benefit to teeth from water fluoride.' However Colquhoun asserts that in all of the studies published in dental professional journals there is bias in population selection and examiner diagnosis and that 'most of the examiners were keen fluoridationists'.

It was my experience in recruiting and standardizing examiners for the California Oral Health Needs Assessment of Children, 1993-94, that none of the examiners could be described as 'keen fluoridationists'.

To overcome the potential of bias on the part of examiners, a study in England transported children from fluoridated and nonfluoridated communities to the examination site and had children wear a smock over their school uniforms so that the examiners would be 'blind' to where the children came from. That study found a benefit of fluoridation in the prevention of tooth decay in children who had been exposed to fluoridation for the first time after the age of 12 years. That study demonstrated that fluoridation does not have to be consumed from infancy to be of benefit. (Hardwick JL, Teasdale J, Bloodworh G. Caries increments over 4 years in children aged 12 at the start of water fluoridation. *British Dental Journal 153* (6) 217-222 1982).

This, and other studies have shown that fluoridation of water supplies exerts a benefit systemically as well as topically. The salivary concentration of fluoride is

higher in fluoridated areas, and the fluoride in saliva exerts a topical effect on teeth to remineralize the enamel to prevent tooth decay.

However, Colquhoun asserts that fluoridation is of 'little if any value' and 'there is negligible benefit from swallowing fluoride'.

7. Colquhoun in discussing fluorosis states: 'Some of these children with these teeth had used fluoride toothpaste and swallowed much of it. But I could not find children with this kind of fluorosis in the nonfluoridated parts of my Health District, except in children who had been given fluoride tablets at the recommended dose at the time.'

In this matter there is general consensus that recent increases in dental fluorosis are attributed to fluoride toothpaste being swallowed by very young children and by fluoride tablets being originally at too high a dose and also being inappropriately prescribed or ingested more frequently than recommended. As a consequence, recommendations have been made to limit the amount of fluoride toothpaste used to a 'pea-size' amount and a lowering of the recommended dose of fluoride supplementation in nonfluoridated communities.

However the influence of fluoride in water fluoridation as being harmful in this way is not generally accepted.

8. The evidence on the role of water fluoridation and hip fracture has been the subject of a number of reviews. 'With respect to hip fractures and bone health, there is no scientific evidence for altering current public health policy on the use of fluorides for caries prevention'. (WHO Expert Committee on Oral Health Status and Fluoride Use. *Fluorides and Oral Health*, WHO technical report series #846, World Health Organization. 1994).

A 1993 review for the U.S. National Research Council addressed fluoride and bone fractures. Of six epidemiological studies using geographic comparison with no actual fluoride intake data, four found a weak association. Of two additional studies examining before and after fluoridation data, one found a negative association and the other no association. One additional essentially geographic comparison found increased risk of hip fracture at 4 mg/L and another where individual exposure data were collected showed no difference in risk.

With regard to animal studies, 'the subcommittee concluded that the weight of evidence indicates that bone strength is not adversely affected in animals that are fed a nutritiously adequate diet unless there is long-term ingestion of fluoride at concentrations of at least 50 mg/L of drinking water or 50 mg/kg in diet.' (National Academy Press: *Health Effects of Ingested Fluoride*; Subcommittee on Health Effects of Ingested Fluoride; Committee on Toxicology, National Research Council; 1993.)

Colquhoun, in his review of fluoride and hip fractures, points to studies published in 1989-90 indicating high doses of fluoride used to treat osteoporosis 'actually caused more hip fractures.'

In recognition of the results of those studies, a lowered protocol of fluoride was used with successful results, which Colquhoun has not included in his 1997

publication. (CYC Pak *et al.* Slow-release sodium fluoride in the management of postmenopausal osteoporosis. Annals of Internal Medicine. April 15, 1994 Vol 120: No. 8. p 625.) These authors concluded that their regimen of 'intermittent slow-release sodium fluoride plus continuous calcium citrate, administered for about 2.5 years, inhibits new vertebral fractures, increases the mean spinal bone mass without decreasing the radial shaft bone density, and is safe to use.' No patient in either experimental or control group developed microfractures, or blood loss anemia. These patients received 25 mg slow-release NaF twice daily in repeated 14 month cycles of 12 months on and 2 months off treatment, compared to a placebo, with both groups receiving 400 mg calcium twice daily.)

In recognition of the study by Pak *et al*, the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIM) issued a press release stating: This regimen (CYC Pak *et al.* Slow-release sodium fluoride in the management of postmenopausal osteoporosis. Annals of Internal Medicine. April 15, 1994 Vol 120: No. 8. p 625.) supports the use of fluoride at high doses for this condition, but has no bearing on fluoridation of water supplies. It adds weight to the hypothesis that there is no positive association between fluoride intake and bone fracture. (National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIM): Fluoride offers hope for treating osteoporosis. Press Release. April 14, 1994.)

9. With regard to studies in Finland, the researchers who had examined autopsy samples of the anterior iliac crest in 1980 and found the highest fluoride content of bone ash from women with severe osteoporosis, also found in 1986 that hip fracture incidence was <u>not</u> affected by fluoridation (Arnala I, Alhava EM, Kivivuori R, Kauranen P. Hip fracture incidence not affected by fluoridation. Osteofluorosis studied in Finland. *Acta Orthopaedica 57* (4) 344-349 August 1986). However, Colquhoun did not include this later study in his publication.

Abstract: Iliac crest biopsies were taken from patients with hip fracture from a low-fluoride area (less than 0.3 ppm), from an area with fluoridated drinking water (1.0-1.2 ppm), and from a high-fluoride area (greater than 1.5 ppm). Fluoride content analysis and histomorphometry of bone were performed. The hip fracture incidence during 1972-1981 was studied in the same areas. The fluoride content of the bone samples correlated with drinking water fluoride. In patients with hip fracture, both osteomalacia and osteoporosis were common. In the high-fluoride area also osteofluorosis was found in many patients. Osteofluorosis may occur if the fluoride content of trabecular bone exceeds 4,000 ppm and either the volumetric density of osteoid or osteoid-covered trabecular bone surface is abnormally increased. There was no difference in incidence of hip fracture in the three areas.

10. A 1996 review on water fluoridation and osteoporotic fractures is included here as background on this subject: Hillier S, Inskip E, Coggon D, Cooper C. Water fluoridation and osteoporotic fracture. *Community Dental Health* Suppl 2 63-68 September 13 1996.

Abstract: Osteoporotic fractures constitute a major public health problem. These fractures typically occur at the hip, spine and distal forearm. Their pathogenesis is heterogenous, with contributions from both bone strength and

trauma. Water fluoridation has been widely proposed for its dental health benefits, but concerns have been raised about the balance of skeletal risks and benefits of this measure. Fluoride has potent effects on bone cell function, bone structure and bone strength. These effects are mediated by the incorporation of fluoride ions in bone crystals to form fluoroapatite, and through an increase in osteoblast activity. It is believed that a minimum serum fluoride level of 100 mg/ml must be achieved before osteoblasts will be stimulated. Serum levels associated with drinking water fluoridated to 1 ppm are usually several times lower than this value, but may reach this threshold at concentrations of 4 ppm in the drinking water. Animal studies suggest no effect of low-level (0-3 ppm) fluoride intake on bone strength, but a possible decrease at higher levels. Sodium fluoride has been used to treat established osteoporosis for nearly 30 years. Recent trials of this agent, prescribed at high doses, have suggested that despite a marked increase in bone mineral density, there is no concomitant reduction in vertebral fracture incidence. Furthermore, the increase in bone density at the lumbar spine may be achieved at the expense of bone mineral in the peripheral cortical skeleton. As a consequence, high dose sodium fluoride (80 mg daily) is not currently used to treat osteoporosis. At lower doses, recent trials have suggested a beneficial effect on both bone density and fracture. The majority of epidemiological evidence regarding the effects of fluoridated drinking water on hip fracture incidence is based on ecological comparisons. Although one Finnish study suggested that hip fracture rates in a town with fluoridated water were lower than those in a matching town without fluoride, a later study failed to show differences. Ecological studies from the United States and Great Britain have, if anything, revealed a weak positive association between water fluoride concentration and hip fracture incidence. Two studies examining hip fracture rates before and after fluoridation yielded discordant results, and are complicated by underlying time trends in hip fracture incidence. Only two studies have attempted to examine the relationship between water fluoride concentration and fracture risk at an individual level. In one of these, women in as high fluoride community had double the fracture risk of women in a low fluoride community. In the other, there was no relationship between years of fluoride exposure and incidence of spine and non-spine fractures. In conclusion, the epidemiological evidence relating water fluoridation to hip fracture is based upon ecological comparisons and is inconclusive. However several studies suggest the possibility of a weak adverse effect, which warrants further exploration. Data on the relationship between fluoride intake and hip fracture risk at the individual level, and data relating fluoridation to bone mineral density are required. Until these become available , the burden of evidence suggesting that fluoridation might be a risk factor for hip fracture is weak and not sufficient to retard the progress of the water fluoridation programs.

11. A 1997 review on osteoporosis, prevention, diagnosis and management is included here as background to this subject: Deal CL. Osteoporosis, prevention, diagnosis and management. American Journal of Medicine 102 (1A) 35S-39S January 27 1997.

Abstract: Osteoporosis is a public health scourge that is usually eminently preventable. Some risk factors, such as low calcium intake, vitamin D deficiency, and physical inactivity, are amenable to early interventions that will help maximise peak bone density. Other risk factors subject to modification are cigarette smoking and excessive consumption of protein, caffeine, and alcohol. Hip fractures are the most serious outcome of osteoporosis, with enormous personal and public health consequences. The ongoing Study of Osteoporotic Fractures has identified additional independent predictors of hip fracture risk, including maternal hip fractures, absence of significant weight gain since age 25, height, hyperthyroidism, use of long-acting benzodiazepines or anticonvulsants, spending < 4 hours per day on one's feet, inability to rise from a chair without using one's arms, poor visual depth perception and contrast sensitivity and tachycardia. In an individual perimenopausal woman, the risk of osteoporotic fracture and the urgency of oestrogen replacement therapy can be best estimated on the basis of bone mineral density as measured by dual-energy x-ray absorptiometry, coupled with the presence or absence of existing fractures and clinical risk factors evident from the history and physical examination. Estrogen, calcitonin and bisphosphonates have all proved effective in retarding postmenopausal bone loss and therefor reducing the risk of fracture. The use of sodium fluoride is more controversial, although a recent study has suggested a possible role for slow-release fluoride combined with high-dose calcium supplementation.

12. Colquhoun used a reference to state: Five years ago, animal experiments were reported of a fluoride-related incident of a rare bone cancer, called osteosarcoma, in young male rats. However that reference did not show any link between fluoride and cancer. It appears that there must have been an error in the publication of Colquhoun's paper.

The cited reference is: Maurer JR; Chang MC; Soysen AG; Anderson RL. Two-year carcinogenicity study of sodium fluoride in rats (see comments). *Journal of the National Cancer Institute* 82 (13) 1118-1126 July 4 1990.

Abstract: To determine the carcinogenic potential of sodium fluoride (NaF), we fed Sprague-Dawley rats a diet containing NaF for up to 99 weeks. Rats receiving NaF at a dose of 4, 10, or 25 mg/kg per day added to a low-fluoride diet were compared with controls receiving either a low-fluoride diet or laboratory chow. Each treatment group consisted of 70 rats of each sex. A 30% decrement in weight gain occurred at an NaF dose of 25 mg/kg per day. Evidence of fluoride toxicity was seen in the teeth, bones, and stomach, and the incidence and severity of these changes were related to the dose of NaF and the duration of exposure. Despite clear evidence of toxicity, NaF did not alter the incidence of preneoplastic and neoplastic lesions at any site in rats of either sex. Results from this study indicate that NaF is not carcinogenic in Sprague-Dawley rats.

The 'equivocal' finding that Colquhoun refers to is of a different rat study by the National Toxicology Program, and the designation 'equivocal' was based on the findings and not on any potential importance to humans.

Colquhoun's interpretation that: 'But now it has been found that the same rare bone cancer has increased dramatically in young human males - teenage boys aged nine to 19 - in the fluoridated areas of America but not in the unfluoridated areas' is different from that of the authors of that study. Indeed the authors of the New Jersey study referenced by Colquhoun stated: 'Therefore, taking both studies together (with reference to the other study by Hoover RN, Devesa S, Cantor K and Fraumeni JF. Time trends for bone and joint cancers and osteosarcoma in the surveillance, epidemiology and end results (SEER) program. In: *Review of Fluoride. Benefits and Risks.* Appendix F. U.S. Department of Health and Human Services, Public Health Service. Washington DC. 1991.) there is insufficient basis to draw conclusions about whether osteosarcoma incidence and fluoridation are causally linked.' (FD Cohn. *An epidemiologic report on drinking water and fluoridation. Environmental Health Service.* New Jersey Department of Health, November 1992.)

Another reference that Colquhoun did not include, perhaps because it didn't support his thesis, is: Gelberg KH; Fitzgerald EF; Hwang SA; Dubrow R. Fluoride exposure and childhood osteosarcoma; a case control study. *American Journal of Public Health 83* (12) 1678-1683 December 1995.

Abstract: OBJECTIVES. This study tests the hypothesis that fluoride exposure in a nonoccupational setting is a risk factor for childhood osteosarcoma. METHODS. A population-based case-control study was conducted among residents of New York State, excluding New York City. Case subjects (n = 130) were diagnosed with osteosarcoma between 1978 and 1988, at age 24 years or younger. Control subjects were matched to case subjects on year of birth and sex. Exposure information was obtained by a telephone interview with the subject, parent or both. RESULTS. Based on the parents responses, total lifetime total lifetime fluoride exposure was not significantly associated with osteosarcoma among all subjects combined or among females. Protective trends were observed for fluoridated toothpaste, fluoride tablets, and dental fluoride treatment among all subjects and among males. Based on the subjects responses, no significant associations between fluoride exposure and osteosarcoma were observed. CONCLUSIONS. Fluoride exposure does not increase the risk of osteosarcoma and may be protective in males. The protective effect may not be directly due to fluoride exposure but to other factors associated with good dental hygiene. There is also biologic plausibility for a protective effect.

13. Additionally, only high levels of fluoride have been associated with reductions in testosterone levels in contrast to Colquhoun's assertion that 'very low levels can interfere with the male hormone testosterone.'

14. With regard to studies from China that children with dental fluorosis have on average lower intelligence scores, Colquhoun misstates the facts of the research. In the study by Li (Li. Effect of fluoride exposure on intelligence in children. *Fluoride 28* (4) pages 189-192 1995) there were four communities with a Community Fluorosis Index (CFI) of <0.4, 0.8, 2,5, and 3.2. Only in the two communities with the highest CFI were lower IQs found. This CFI index was developed by Dean (Dean HT. The investigation of physiological effects by the

epidemiological method. In: Moulton FR (Ed). *Fluorine and Dental Health.* Am Assoc Adv Sci, Washington 1942 pp 23-31). Dean stated that a CFI below 0.4 is of little or no public health concern; that the range from 0.4 to 0.6 is borderline; and that for indexes above 0.6 removal of excess fluoride from the water is recommended. Thus the communities where Li found lower intelligence scores were in areas of exceptionally high fluoride, apparently due to high fluoride coal being burned and inhaled, rather than in the water. Thus these findings are unrelated to water fluoridation and to suggest, as Colquhoun does, that children with dental fluorosis have on average lower intelligence scores is a gross misstatement of the facts, since there are children with very mild and mild dental fluorosis, living in the low CFI communities where IQ scores were apparently normal.

15. Colquhoun supports his contention that fluoride is harmful by citing the reference by Mullenix *et al.* (Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. Neurotoxicity of sodium fluoride in rats. *Neurotoxicology and Teratology 17* (2) 169-177 March-April 1995.) In this study pregnant rats were injected with very high doses of sodium fluoride at 0.13 mg/kg. This would be equivalent of a human dose for a pregnant woman of 6.5 mg, not to be swallowed and therefore diluted by the body, but injected subcutaneously. This would be unconscionable in humans who are never injected with sodium fluoride. Then the weanlings (baby rats) drank water with 0, 75, 100, or 125 ppm F for 6 or 20 weeks, and the 3 month-old adults received water containing 100 ppm F for 6 weeks. Therefore all rats drank water with at least 100 times the recommended concentration for water fluoridation for extended periods of time. Thus this study was never intended to determine the effects of water fluoridation.

16. Colquhoun consistently alleges harm from fluoride without stating the dose or concentration of fluoride, which is absolutely essential in a discussion of the toxicology of fluoride.

This concludes my review.

RESPONSE TO CRITIQUE OF HOWARD POLLICK

John Colquhoun

Pollack argues mainly by quoting fluoridationist opinions, rather than evidence. I here respond to each of his 16 points:

1. What I actually wrote was: "Over the next few years these treatment statistics, collected for all children, showed that when similar fluoridated and nonfluoridated areas were compared, child dental health continued to be slightly better in the nonfluoridated areas [5,6]." The two references, from peer-reviewed journals, presented dental health data for the entire child population of (a) Greater Auckland, containing a quarter of New Zealand's population, and (b) the main population centers of New Zealand. Unfortunately, the last "nonfluoridated" word was misprinted as "fluoridated", quite altering the sentence's meaning. I have requested that a correction be published. If Pollick had read the two references he would have realized the sentence contained a misprint.

2. Pollick alleges I was "misleading" when I stated that the pro-fluoridation report of US Public Health Service authors Brunelle and Carlos was "apparently intended to counter" the Yiamouyiannis finding of no benefit from fluoridation. Yiamouyiannis' criticism of the Brunelle and Carlos paper was published as an addendum, when their attempt to refute the "no-benefit" finding had appeared. Obviously the fact that Yiamouyiannis had obtained the data showing no benefit from the US Public Health Service, using the Freedom of Information Act, would be known to Brunelle and Carlos when they prepared their paper. So my statement was not misleading.

3. Pollick alleges I was biased because I did not record the titles of profluoridation authors. When I listed the well-known fluoridationists whom I visited, early in my paper, no slight was intended. The content made clear that they were leading profluoridation experts.

4. Teotia's studies in India included low as well as high water fluoride areas.

5. Pollick agrees that nutrition is related to dental disease in developing countries, and offers no reason why the same should not apply in developed countries where, in poverty stricken areas, all diseases, including dental disease, are more prevalent than in affluent areas.

6. Pollick simply asserts his belief that fluoridation exerts both a systemic and topical dental benefit, but is unable to produce any study which counters my statement: "It is just not possible to find a blind fluoridation study in which the fluoridated and nonfluoridated populations were similar and chosen randomly."

7. Again, Pollick simply asserts his opinion (claiming "general consensus") that the fluoride in fluoridated water somehow does not cause dental fluorosis while the fluoride in swallowed toothpaste and tablets does. In my paper I cited several studies which have reported higher fluorosis prevalences in fluoridated areas.

8. On the subject of hip fractures, Pollick quotes opinions expressed in "reviews" of pro-fluoridation authorities, but does not answer the points I made in my paper. His defense of high doses of fluoride for attempts to treat osteoporosis (citing the controversial Pak *et al* paper) is not an opinion shared by many other clinicians.

9. Pollick criticizes my failure to include, among my 73 references, a 1986 paper which reported no association between water fluoride and hip fractures. The paper was published when it was still being claimed that fluoride reduced hip fractures. Since then, the much more comprehensive studies reporting the association between hip fractures and fluoridation have been published.

10. The opinion, "the burden of evidence suggesting that fluoridation might be a risk factor for hip fracture is weak and not sufficient to retard the progress of the water fluoridation program", is also not shared by many other scientists.

11. It is difficult to see how an opinion on "possible role for slow-release fluoride combined with high-dose calcium supplementation" is related to the issue of mandatory fluoridation.

12. I agree that the National Toxicology Program study should be cited rather than the Maurer *et al* study, but that is a minor point. Pollick's quoting of the opinion that "there is insufficient basis to draw conclusions about whether osteosarcoma incidence and fluoridation are causally linked" does not alter the facts I presented: *viz.* Animal experiments, showing the rare bone cancer, osteosarcoma, occurred in male rats after fluoride ingestion were followed by reports of increased osteosarcoma in young human males in fluoridated areas but not in nonfluoridated areas.

Pollick asserts "Another reference that Colquhoun did not include, perhaps because it didn't support his thesis, is ..." I not only cited the Gelberg *et al* study (reference 61) but also discussed its glaring faults (see p 114, p 40 in original).

13. The study I cited (Kanwar *et al* 1983) reported reduced testosterone levels at very low as well as high levels of fluoride.

14. Pollick alleges that I "grossly" misstated the facts of Chinese research on intelligence. A reading of the research will confirm the accuracy of my statements.

15. Pollick argues that, because only high intakes of fluoride caused intelligence deficits in rats in the Mullenix experiment, therefore the finding is unrelated to the issue of water fluoridation. Like most fluoridationists, he ignores the possibility that (as has been acknowledged with lead and other toxins) low intakes could have similar deleterious long term effects to the short term ones resulting from high intakes.

16. The same observation applies. However, the statement that I ignored "the dose or concentration of fluoride" comes strangely from one who advocates a measure which supplies an uncontrolled dose (depending on amount of water consumed) to entire populations.

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