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ABSTRACTS

On dental effects:

On effects on bone:

FLUORIDE - MAGNESIUM INTERACTION

A Machoy-Mokrzynska. Szczecin, Poland

It has now been fifteen years since Marier drew attention to the significance of magnesium in biological interaction with fluorides.[1] The toxic effect of fluoride ion plays a key role in acute Mg deficiency. The amount of F^- assimilated by living organisms constantly increases, and Mg absorption diminishes as a consequence of progressively advancing industrialization. Marier gives examples of such retention of both elements in plants (*eg* in pine and tomatoes) and in animals, for instance in bone tissue, blood and kidneys, with the last being thought as the most probable place of Mg-F^- interaction.[1] Now, further facts have been observed, which throw a new light on the effects of Mg- F^- interaction.

The significance and distribution of Mg in living organisms are widely known and described in textbooks.[2] Fluoride ion clearly interferes with the biological activity of magnesium ion.[3] Present-day Mg deficiencies in humans are the result of intensive expulsion of this element (*eg*, under the influence of extensive drinking of alcoholic beverages) or reduced Mg content in the diet, caused, for example, by inappropriate agricultural practices or effects of ecotoxins.[4]

One of the prime locations of possible F^- and Mg interactions is the intestines. The increased F^- supply reduces intenstinal Mg resorption, owing to high chemical affinity of both elements and production of MgF^+ and MgF_2.[1] However, there are many facts to be considered, since there is a common mechanism of transportation of both these elements through the intestinal walls. Distinct F^--Mg interaction is also observed in other cells and tissues. Mg deficiency in plants may limit synthesis of chlorophyll, on which photosynthesis depends. Therefore, supplementation of Mg protects plants against toxic effects of fluoride compounds.[1] Mg deficiency in animals reduces production of energy, relevant to the Mg-ATP system. Reduction of ATP levels affects in an unfavourable way many metabolic processes connected with the action of ATP (*eg*, metabolism of carbohydrates, proteins, nucleic acids, lipids, and active transport).

The role of Mg and F^- ions in enzymology is also well known. Magnesium-dependant enzymes compose the biggest group in enzyme systematics. Magnesium is the activator of more than 300 enzymes, while fluorine is known. as their inhibitor, although the activity of some enzymes is known to be increased by fluorine.[5] In general, Mg-F^- interactions most frequently decrease enzymatic activity.[6] The greatest practical significance of Mg-F^- interaction however, seems to be in processes of bone and tooth mineralization, and in the formation of uroliths.[7,8]

In bone tissue magnesium stimulates the transformation of immature (amorphic) bone into a more crystalic form. Owing to the translocation of Mg into mineral tissue, bone elasticity increases to help prevent fractures. Rats on diet poor in Mg display significantly higher content of F^- in femurs and molars. This is undoubtedly related to the assimilability of both elements. Since bioavailability of Mg and F^- depends on their mutual ratio in the diet,[9] a low-magnesium diet distinctly increases F^- absorption in the intestines.

Institute of Pharmacology and Toxicology. Pomeranian Medical Academy. al. Powstanców Wlkp. 72. 70 111 Szczecin. Poland.

Taking into account the mineralization of bone tissue, one also cannot ignore the role of calcium. The basic inorganic compound of bones is hydroxyapatite, containing calcium phosphate. The far-reaching antagonism between magnesium and calcium affects not only their different distribution in tissues, but also their mutual dislodging from cells. For example, magnesium favours blocking of calcium channels, disturbs oxidative phosphorylation, intensifies bone decalcification and increases muscle-cell diastole, while calcium intensifies contraction. On the other hand, hypercalcemia enhances Mg loss or magnesiuria.[10]

Mg-F^- interaction in processes relating to enamel and its effect on caries have also been investigated. Fluoride ion affects enamel hardening[11,12] and prevents its annealing, but this effect diminishes after administration of Mg. Magnesium alone does not visibly affect tooth plaque, erosive enamel damage, or the course of caries, but Mg and F^- administered jointly influence enamel hardening and reduce caries significantly, as demonstrated in rats.[13] In interactions of F^- with Mg and Ca, it should be stressed that it is calcium rather than magnesium that intensifies mineralization processes.

Urolith formation is considered to be pathological. Mineral content analysis of uroliths shows that they always contain Mg and F^- (besides phosphates, calcium and other inorganic and organic components).[8] Formation of uroliths follows crystallization rules. Mg ion reduces the rate of superficial crystal nuclei formation, whereas F^- ion accelerates the process. The former reduces and the latter accelerates growth of calcium phosphate crystals.[7] In the formation of uroliths, calcium is the promotor, and magnesium plays the role of the inhibitor.

It also should be pointed out that uroliths always contain more Ca than Mg. Fluoride, on the other hand, favours formation of uroliths and accelerates their production.[8]

In summary, it can be stated that in intoxication with fluorine compounds, magnesium plays a protective role by countering and reducing the toxic effects of F^-.

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XXIst WORLD CONFERENCE

of the INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH
and the HUNGARIAN SOCIETY FOR FLUORIDE RESEARCH
BUDAPEST, HUNGARY. AUGUST 18 - 22, 1996

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KILLING THE MESSENGER

In historic times, the bearer of bad news was in danger of personal demise, as anger at the message was displaced on to the messenger. In 1898, Christy noted the maxim "Messengers should neither be beheaded nor hanged".[1] In the present age such behaviour would not be tolerated but the underlying tendency towards expressing displeasure towards those bringing unwelcomed information may still persist.

A recent article on "Putting Yiamouyiannis into Perspective" appears to focus on the status of John Yiamouyiannis as a serious research worker or commentator on health policy with the suggestion that his work be regarded with considerable scepticism.[2] He is seen to use deception by omission, references which do not support his claims, to be associated with an organization which was a front for promoters of unproved remedies, eccentric theories and quackery, to have had political ambitions and have taken the role of propagandist rather than serious scientist so that his hostility to fluoridation has obscured his scientific judgement. The article suggests that the simple truth is that there is no scientific controversy over the safety of fluoridation. The underlying theme of the article could thus be seen to be "character assassination" rather than a critique of a particular argument.

George Waldbott, a founding member of the International Society for Fluoride Research, noted that he became the subject of a dossier in which he was accused of intellectual dishonesty and incompetence.[3] The dossier became part of a brochure, "Comments on the opponents of fluoridation", which was published twice by the Bureau of Public Information of the American Dental Association.[4,5] Waldbott considered that the dossier was circulated widely when he raised his voice against fluoridation. Although he described the use of double-blind methodology to test for adverse reactions to fluoride,[6] he has been reported to have admitted under cross-examination in 1982 to have not carried out double-blind studies but to have relied instead on personal intuition.[7]

In the debate on fluoridation prior to the referendum in October 1995 in Timaru, New Zealand, reference to the work of Waldbott was seen to represent a "clasp at yet another straw in the desperate attempt to find, this time, a cause and effect relationship between fluoridation and allergy".[8] The author, a public health official, stated that Waldbott "admitted under cross-examination that he had no research training and that his studies were not double blind but relied on personal intuition", and saw that opinion of the researcher rather than a reasoned critique of the research as reason for dismissing the studies.[8]

Leon Festinger in his theory of cognitive dissonance, suggests that two cognitive elements are in a dissonant relation if, considering these two alone, the obverse of one element follows from the other.[9] The presence of dissonance is seen to give rise to pressures to reduce that dissonance. Dissonance introduced by disagreement expressed by other persons may be reduced by changing one's own opinion, by influencing the others to change their opinion, and by rejecting

those who disagree. The other person can be made, in some manner, not comparable to oneself. One can attribute different characteristics, experiences, or motives to the other person or one can even reject him and derogate him. If one person believes that flying saucers are space ships from other planets and some other person voices the opinion that flying saucers, as such, do not even exist, the resulting dissonance in the cognition of the former may be reduced if he can believe that the latter is a stupid, ignorant, unfriendly, and bigoted individual. Festinger indicates that it sometimes happens that a large group of people is able to maintain an opinion or belief even in the face of continual definite evidence to the contrary. He notes that groups of scientists have been known to continue to believe in certain theories, supporting one another in this belief in spite of mounting evidence that these theories are incorrect. Thus it is possible for reality to be denied and personal attacks be made on the character of those presenting alternative incompatible viewpoints.

The effects of fluoride are multiple and complex. In order for a consensus to emerge on these the debate needs to focus on the evidence rather than on the character of those producing the evidence.

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Bruce Spittle

I know that most men, including those at ease with problems of the greatest complexity, can seldom accept even the simplest and most obvious truth if it be such as would oblige them to admit the falsity of conclusions which they have delighted in explaining to colleagues, which they have proudly taught to others, and which they have woven, thread by thread, into the fabric of their lives.

Leo Tolstoy

CORRELATION AMONG HEAVY METALS AND FLUORIDE IN SOIL, AIR AND PLANTS IN RELATION TO ENVIRONMENTAL DAMAGE

N P Gritsan,[1] G W Miller[2] and G G Schumatkov
Dnepropetrovsk, Ukraine and Logan, Utah, USA

SUMMARY: The investigations were conducted at 46 sites in the South-East part of the Ukraine that had different levels of industrial pollution. Concentrations of fluoride and 16 other macro- and microelements were determined in plants (leaves and seeds) and soils to determine if there were any quantitative relationships between levels of these elements and abnormal plant development. Damage criteria such as the frequency of chromosome aberrations in root tips of *Robinia pseudoacacia* (L) seeds, determination of germination percentage, peroxidase activity of leaves and soils and quantity of microfauna were studied. It was found that among the environmental pollutants, fluoride was most damaging.

Key words: Environmental pollution; Fluoride; Chromosome aberrations, *Robinia pseudoacacia* (L), Ukraine.

Introduction

At present in the Dnepropetrovsk region, a rather complicated ecological situation has been created as a result of technogenic activity. The main pollutants in this region come from metallurgical and chemical industries and from thermal power stations. Concentrations of harmful substances in plants and soils were on the average from 2-20 times higher than in unpolluted areas. The type of pollution in sampling areas depended mainly upon the kind of industry. For example, plant and soil samples collected from the vicinity of the biggest non-ferrous metallurgy plant in Europe had extremely high levels of fluoride. Thus consequences of the intensive development of industry are most damaging for the environment in Dnepropetrovsk. The negative impact of industry leads to changes in chemical composition of the biosphere, accumulation of pollutants in soils, plants, water bodies and food products. Consequently it has caused a negative impact on human health. To understand and prevent future impacts of industries on the environment it is necessary to study the relationships between heavy metal, fluoride content and abnormal plant development.

To accomplish this damage criteria such as the frequency of chromosome aberrations in root tips of *Robinia pseudoacacia*, determination of germination percentage, chlorophyll content, peroxidase activity of leaves and soils, ash of soils and plants and quality and quantity of soil microfauna (*Collembola*) were studied.

Materials and Methods

The investigations were conducted in the South-East part of the Ukraine where different levels of industrial pollution were found. The Rowan tree (*Robinia pseudoacacia* (L)) was chosen as a test culture for this study. The plants and soils were sampled in the contaminated Dnepropetrovsk region. The area used for

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[1] Institute of Nature Management Problems and Ecology, Ukrainian Academy of Sciences, Dnepropetrovsk, Ukraine. [2] Utah State University, Logan, Utah, USA. Presented to the XXth Conference of the International Society for Fluoride Research, Beijing, September 5-9, 1994.

control was situated 60 km from Dnepropetrovsk and was free of industrial pollution. Therefore it was possible at both sites to collect plants and soils for comparison. The soil type of the studied region was mainly chernozem. The frequency of chromosome aberrations in root tips of seedlings was determined using well-known techniques to study the genetic consequences of anthropogenic impact on plants.[1] Determination of germination percentage, chlorophyll content, and peroxidase activity of leaves and soils were determined using methods described by Pleshkov.[2] Atomic absorption spectrophotometry was used for heavy metal determinations in plants and soils.[3]

The samples were analyzed for fluoride using a fluoride selective ion electrode.[4]

Coefficients of contamination (C_c), deficiency (C_d), unbalance (C_{un}) and index of ecological stress were elaborated for environmental assessment by Gritsan.[5]

Coefficients of contamination (and deficiency for one element) were determined using the following models

$$1)\quad C_c = \frac{C\ found}{C\ Normal} \qquad 2)\quad C_d = 1 - \frac{C\ found}{C\ Normal}$$

Total coefficient of contamination (C_c) and total coefficient of deficiency C_d were proposed for environmental assessment under conditions of contamination by several elements.

$$\underline{C}_c = \frac{1}{n} \sum_{I=1}^{n} \left(\frac{c}{C_N} - I\right) \qquad C_d = \frac{1}{n} \sum_{I=1}^{n} \left(1 - \frac{C}{C_n}\right)$$

Coefficient of unbalance was determined as $C_{un} = \underline{C}_c + \underline{C}_d$ for each region.

Results and Discussion

Annually 280 thousand tons of harmful substances are emitted into the air of the Dnepropetrovsk region (among them two tons of lead and seven tons of fluoride). Average concentration of dust, SO_2, CO, N_xO_x, SH_2, phenol and NO_3 in the air were 0.2, 0.01, 1.7, 0.04, 0.04, 0.003, 0.03, mg/m^3, respectively. Total coefficients of contamination were 710 (dust), 2.0 (SO_2), 12 (CO) and 40 (N_xO_x). The main pollutants in air contamination among the heavy metals were copper and nickel (Table 1A). The investigations showed considerable accumulation of Fe and Ni by plants in the Dnepropetrovsk region. During the vegetation period accumulation of Cr, Zn, Pb, Cu, and Co by plants (Table 1B) and Zn, Pb, Na, Cd, Ni, Cu, Cr, and Fe by soils was observed (Table 1C). Total coefficients of contamination and unbalance are presented in Table 2. Coefficients of contamination for soils and plants are identical. The deviation from the control of the main biological indices are given in Table 3. It was established that increases in environmental toxicants lead to increases in the mutability of living organisms. The percentage of mutation in the meristematic cells of root tips of seed (*Robinia Pseudoacacia*)[2] growing in polluted areas was 15 time higher than the control (Table 2).

There is considerable evidence in the literature that many ecosystems are affected by industrial pollution. This study was carried out to investigate and determine

the most harmful toxic element in land ecosystems of the Dnepropetrovsk region. In Table 4 the coefficients of correlations between element concentration in plants and soils and biological indices are shown.

A strong positive correlation between fluoride content and chromosome aberrations was found in this study. Coefficient of correlation between the fluoride content and peroxidase activity in the plants was +0.63. Our investigations showed negative correlations between chlorophyll and fluoride content. Coefficient of correlation was -0.55. The same negative relationship between the fluoride content and ecosystem criteria was established. The relationships between fluoride content in plants and abnormal plant development are presented as regression equations (Table 5).

The relationship between fluoride and other elements both in plants and in soils was investigated. Positive correlation between fluoride and copper was determined in plant and soils. Coefficients of correlation were 0.67 and 0.76, respectively (Tables 5, 6, 7). The middle positive correlations between fluoride and Zn ($r = 0.62$) and Fe ($r = 0.68$) in leaves were found. As to relationships the data of the study showed that the percentage of germinated seeds in polluted areas was 25.61% compared to 58.88% in the control area. Therefore, the studied plant populations of contaminated areas were probably not able to reproduce satisfactorily for self-restoration. The ash of plants and soils is the most important index for assessment of environmental contamination by metals. In contaminated areas the ash of leaves and soils was increased 5% and 17% respectively, as compared to controls (Table 2).

Table 1A. Content of metals in air

Elements	Air average data, mg / kg		
	Dnepropetrovsk	Control	Coeffic. cont. (Cc)
Mg			
Cr	0.016	0.0008	20
Mn	0.24	0.03	8.0
Fe			
Co	0.01	0.0008	13.8
Ni	0.10	0.004	25.0
Cu	0.39	0.004	98
Zn	0.34	0.03	4.3
Cd			
Pb	0.08	0.05	1.6
Al			
Ca			
Ag			
Mo			
K			
Na			
Total coef. of contamination		24.37	

Table 1B. Content of metals in leaves of *Robinia pseudoacacia* plants

Elements	Leaves average data, mg/kg		
	Rural	Dnepropetrovsk	Control
Mg	4160	4550	3600
Cr	12	16	10
Mn	146	93	10
Fe	8380	1960	900
Co	7	8	6
Ni	14	8	9
Cu	10	14	9
Zn	19	36	16
Cd	1	1	1
Pb	11	13	10
Al	640	1056	500
Ca	25790	33710	21000
Ag	1	1	1
Mo	20	20	20
K	11430	10100	11400
Na	630	550	1300

Table 1C. Content of metals in soil

Elements	Soils average data, mg / kg		
	Rural	Dnepropetrovsk	Control
Mg	4760	4000	3600
Cr	39	42	34
Mn	579	749	500
Fe	20870	26220	16170
Co	12	10	10
Ni	17	16	13
Cu	19	30	13
Zn	66	128	41
Cd	1	1	1
Pb	16	30	10
Al	32360	27356	30000
Ca	15030	45180	10000
Ag	1	1	1
Mo	20	20	20
K	13050	10520	8600
Na	4270	4500	2800

A considerable increase of peroxidase activity and a decrease in chlorophyll content was also found in the polluted area.

Between F and Cd, Cr, Co, Zn, and Pb in soils, positive correlations were established (Table 6). Our study shows that high levels of F-accumulation in plants was accompanied by high concentrations in the soil of such elements as Cu, Cr, Zn, Ca, and F (Table 7).

In Table 8 correlations between the soil elements and plant elements are presented. Highest correlations are shown between Cr in the soil and Fe in the plant, Fe in the soil and the plant, Cu in the soil and the plant, Cu in the soil and F in the plant, K in the soil and Mg in the plant. There was also a high correlation between F in the soil and F in the plant. Regardless of this correlation, the F accumulation in the leaves probably came from the air. Of the investigated elements, fluoride is one of the most harmful pollutants for land ecosystems of the South-Eastern part of the Ukraine, and this problem requires close attention and further study.

Table 2. Environmental assessment according to total coefficient of contamination and unbalance and some biological indices

	Plant leaves	Soil	Coef. of unbalance	% chromo-some aberra-tions	% of germi-nation	Ash %		Plant leaves	
	Cc	Cc	Cun			leaves	soil	Peroxidase activity units/mg protein min	Chlorophyll content mg/g net weight
Average data polluted area (n = 17)	0.63	0.64	1.41	12.48	25.61	8.25	89.20	3.77±0.19	3.33±0.23
Control (unpolluted area)	0.28	0.11	0.54	0.8	58.88	7.89	76.30	2.88±0.12	4.22±0.31

Table 3. Deviation from the control of biological indices

Biological indices	Control area	Contaminated area	Standard deviation from control
Percentage of chromosome aberrations	0.80	12.48	15.5
Peroxidase activity of leaves	2.88	3.77	1.2
Chlorophyll content	4.22	3.33	-1.1
Biomass of 1000 seeds	-	-	1.0
Germination percentage	48.88	25.61	1.8
Quantity of *Collembola*	-	-	-4.1
Peroxidase activity of soil	-	-	-2.8

Table 4A. Correlation between the element concentration in plant and soil in relation to biological criteria

Indices	Mg	Cr	Mn	Fe	Co	Ni	Cu
Plant							
Peroxidase activity	-0.17	0.40	-0.16	0.64*	0.34	-0.11	0.48
Biomass of 1000 seeds	0.52*	0.12	0.04	-0.14	0.40	0.43	0.16
Germination percentage	-0.26	0.27	-0.17	0.18	0.02	-0.17	0.48
Chromosome aberrations	-0.17	0.35	-0.29	0.74**	0.24	-0.20	0.60*
Chlorophyll content	0.28	-0.36	0.12	-0.59*	-0.14	0.20	-0.37
Soil							
Peroxidase activity	-0.20	-0.30	-0.38	-0.31	-0.23	-0.27	-0.43
Quantity of *Collembola*	0.34	0.03	-0.23	-0.10	-0.03	0.25	-0.21

* High correlation
** Highest correlation

Table 4B. Correlation between the element concentration and biological criteria

Indices	Zn	Cd	Pb	Al	Ca	K	Na	F
Plant								
Peroxidase activity	0.40	0.09	0.28	0.08	0.37	-0.17	-0.39	0.63*
Biomass of 1000 seeds	-0.28	0.19	0.16	0.05	0.49	-0.10	-0.10	-0.11
Chromosome aberrations	0.42	0.08	0.26	0.08	0.34	-0.23	-0.23	0.70
Chlorophyll content	-0.49	0.02	-0.14	-0.05	-0.04	-0.03	0.61	-0.55*
Soil								
Peroxidase activity	-0.67*	-0.25	-0.22	-0.10	0.57*	-0.07	-0.04	-0.12
Quantity of *Collembola*	-0.29	0.08	-0.38	0.34	-0.14	0.37	0.12	-0.13

* High correlation
** Highest correlation

Table 5. Relationship between F-content and abnormal plant development

X	Y	Regression equation
F-content in leaves	Chlorophyll content	$Y = 3.71 - 0.004x - 0.0001x^2$
—	Peroxidase activity	$Y = 2.73 + 0.03x - 0.0001x^2$
—	Chromosome aberrations	$Y = -1.73 + 0.27x + 0.002x^2$
—	Germination percentage	$Y = 56.22 + 0.54x - 0.003x^2$
F-content in seeds	Chromosome aberrations	$Y = -3.29 + 0.37x + 0.16x^2$

Table 6A. Correlation between the elements in the soil

	Mg	Cr	Mn	Fe	Co	Ni	Cu
Mg		0.49	0.24	0.08	0.60*	0.75**	0.46
Cr			0.70**	0.71**	0.74**	0.50*	0.65*
Mn				0.84**	0.75**	0.40	0.51*
Fe					0.62*	0.21	0.27
Co						0.58*	0.49
Ni							0.37
Cu							
Zn							
Cd							
Pb							
Al							
Ca							
K							
Na							
F							

*High Correlation
**Highest Correlation

Table 6B. Correlation between the elements in the soil

	Zn	Cd	Pb	Al	Ca	K	Na	F
Mg	0.15	0.07	0.26	0.81**	0.45	0.67*	0.66*	0.40
Cr	0.72**	0.67*	0.64*	0.43	0.34	0.09	0.27	0.61*
Mn	0.68*	0.75**	0.53*	0.14	0.45	-0.15	0.08	0.47
Fe	0.61	0.85**	0.35	-0.02	0.16	-0.16	-0.16	0.46
Co	0.48	0.59*	0.46	0.46	0.47	0.14	0.31	0.61*
Ni	0.21	0.32	0.18	0.55*	0.38	0.53*	0.50*	0.35
Cu	0.73*	0.29	0.79**	0.53*	0.62*	-0.08	0.56	0.67
Zn		0.65*	0.69*	0.20	0.54*	-0.25	0.18	0.58*
Cd			0.34	0.09	0.14	-0.14	0.01	0.57*
Pb				0.21	0.40	-0.23	0.46	0.56*
Al					0.22	0.60*	0.69*	0.48
Ca						0.01	0.34	0.43
K							0.49	0.15
Na								0.44
F								

*High Correlation
**Highest Correlation

Table 7A. Correlation between the elements in plant leaves

	Mg	Cr	Mn	Fe	Co	Ni	Cu	Zn
Mg		0.04	-0.02	-0.24	0.46	0.05	0.02	-0.19
Cr			-0.11	0.16	0.22	0.31	0.43	0.76**
Mn				-0.15	0.25	0.03	-0.19	-0.15
Fe					0.27	-0.10	0.33	0.29
Co						0.17	0.01	0
Ni							0.17	0.18
Cu								0.53*
Zn								
Cd								
Pb								
Al								
Ca								
K								
Na								
F								

*High Correlation
**Highest Correlation

Table 7B. Correlation between the elements in plant leaves

	Cd	Pb	Al	Ca	K	Na	F
Mg	0.40	0.35	-0.30	0.52*	-0.41	-0.15	0.05
Cr	-0.19	-0.07	0.32	0.17	0.12	-0.40	0.42
Mn	0.03	-0.08	0.26	0.11	0.14	-0.43	-0.11
Fe	-0.01	0.08	0.02	0.40	-0.23	-0.22	0.68*
Co	0.75**	0.75**	-0.05	0.62*	-0.46	-0.45	0.26
Ni	0.02	0.01	0.56*	-0.12	0.37	-0.12	-0.03
Cu	-0.19	0.03	0.42	0.20	0.02	-0.23	0.76**
Zn	-0.20	-0.13	0.27	-0.13	0.36	-0.33	0.62*
Cd		0.90**	-0.11	0.24	-0.47	-0.07	-0.02
Pb			0.02	0.34	-0.60*	-0.12	0.08
Al				-0.12	0.12	-0.13	0.13
Ca					-0.71**	-0.27	0.34
K						-0.02	-0.12
Na							-0.44
F							

*High Correlation
**Highest Correlation

Table 8. Correlation between the elements in plants

	Mg	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Al	Ca	K	Na	F
Mg	0.33	0.04	-0.29	0.28	0.31	0.16	0.40	0.27	0.13	0.12	-0.04	0.40	-0.11	0.02	0.36
Cr	-0.18	0.42	-0.53*	0.73**	0.15	0.07	0.48	0.36	-0.07	0.02	0	0.20	-0.05	-0.14	0.56*
Mn	-0.20	0.13	-0.13	0.59*	0.07	0.17	0.22	0.12	-0.02	-0.11	-0.14	0.16	0.08	-0.12	0.37
Fe	-0.20	0.20	-0.17	0.71**	0.11	-0.16	0.03	0.11	-0.12	-0.15	-0.22	0.21	0	-0.17	0.34
Co	0	0.03	-0.26	0.68*	0.20	-0.07	0.27	-0.08	0	0.03	-0.14	0.49	-0.24	-0.03	0.33
Ni	0.38	0.39	-0.25	0.25	0.24	-0.01	0.37	0.20	0.05	-0.03	-0.14	0.44	-0.23	-0.21	0.48
Cu	-0.22	0.37	-0.29	0.57*	0.04	-0.11	0.79**	0.56	-0.07	0.08	0.24	0.20	-0.03	-0.10	0.71**
Zn	-0.24	0.40	-0.14	0.66*	0.28	0.08	0.57*	0.47	0.02	0.12	0.10	0.18	0.13	-0.39	0.68*
Cd	-0.04	0.16	-0.31	0.55*	0.14	-0.13	0.15	-0.04	-0.08	-0.08	-0.31	0.30	-0.11	-0.01	0.35
Pb	-0.41	0.22	-0.15	0.57*	0.19	-0.16	0.42	0.21	0.07	0.22	0.19	0.24	-0.17	0.11	0.35
Al	0.32	0.37	-0.47	0.13	-0.01	0.11	0.61*	0.30	-0.13	0.02	0.06	0.28	-0.09	0.25	0.31
Ca	0.09	-0.13	0.04	0.47	0.33	-0.07	0.50*	0.22	0.36	0.30	-0.04	0.20	-0.01	-0.28	0.64*
K	0.81**	0.30	-0.25	-0.20	0.36	0.20	0.06	0.03	0.26	0.25	-0.19	0.39	-0.24	-0.09	-0.03
Na	0.39	0.28	-0.11	0.04	0.33	-0.02	0.58*	0.07	0.21	0.32	0.19	0.55*	-0.38	0	0.28
F	0.14	0.14	-0.57*	0.59*	0.20	-0.34	0.43	0.17	0.11	0.29	-0.32	0.54*	-0.44	0.05	0.51*

* High correlation ** Highest correlation

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EFFECT OF FLUORIDE EXPOSURE ON INTELLIGENCE IN CHILDREN

X S Li,[a] J L Zhi,[b] and R O Gao[c]
Guizhou, China

SUMMARY: The intelligence was measured of 907 children aged 8-13 years living in areas which differed in the amount of fluoride present in the environment. The Intelligence Quotient (IQ) of children living in areas with a medium or severe prevalence of fluorosis was lower than that of children living in areas with only slight fluorosis or no fluorosis. The development of intelligence appeared to be adversely affected by fluoride in the areas with a medium or severe prevalence of fluorosis but to a minor extent only in areas with only a slight prevalence of fluorosis. A high fluoride intake was associated with a lower intelligence. No correlation was found between age and intelligence in the areas with a medium and severe prevalence of fluorosis. The effect of exposure to a high level of fluoride on intelligence may occur at an early stage of development of the embryo and infant when the differentiation of brain nerve cells is occurring and development is most rapid.

Keywords: Child; China; Fluoride; Fluorosis; Intelligence; Intelligence testing.

Introduction

With the study in recent years of endemic fluorosis, attention has been given to the effect of fluoride on intelligence in children.[1-2] In the present study children living in areas with differing prevalences of fluorosis received intelligence testing.

Materials and Methods

The survey was carried out in November and December 1991 in the Anshu and Zhijin counties in Guizhou Province. The prevalence of fluorosis due to soot from coal burning varied from being absent to being present to a slight, medium or severe degree. In the medium and severe fluorosis areas, it was customary for coal to be used as a domestic fuel for cooking, heating and drying grain whereas in the areas without or only slight fluorosis there was no custom of drying grain by the use of coal. The standards of material and cultural life for the children were similar in all four areas. The survey areas have no iodine deficiency disease.

The children surveyed were of Han nationality and numbered 907 with ages from 8-13 years. Children whose intelligence had been affected by congenital or acquired diseases not related to fluoride were excluded.

Intelligence was measured using the *China Rui Wen's Scaler for Rural Areas*.[3] In each area, 20-24 children were examined, by a special examiner, in each age group with the groups being separated by intervals of six months in age.

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[a] Guizhou Provincial Sanitary and Anti-Epidemic Station, Guiyang City, 550001, China. [b] Anshun Prefecture Sanitary and Anti-Epidemic Station. [c] Zhijin County Sanitary and Anti-Epidemic Station.

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The significance of the various levels in the Intelligence Quotient (IQ) were: <70 low; 70-79 borderline; 80-89 lower medium; 90-109 medium; 110-119 upper medium; 120-129 excellent; >129 special excellence.

The dental fluorosis index using the method of Dean and the urinary fluoride concentrations for the children from the four different areas are shown in Table 1.

Table 1. Dental fluorosis prevalence, dental fluorosis index and urinary fluoride concentration for children from areas with no fluorosis and slight, medium and severe prevalences of fluorosis

	Non-fluorosis area	Slight fluorois area	Medium fluorosis area	Severe fluorosis area
Dental fluorosis index	<0.4	0.8	2.5	3.2
Urinary F mg/L	1.02	1.81	2.01	2.69

Results

The numbers of children examined in each area and their mean IQs are shown in Table 2.

The distribution of the IQ scores is shown in Table 3.

As shown in Table 3, more children with IQs of <70 and 70-79 and fewer children with IQs of 90-109 and 110-119 were present in the medium and severe fluorosis areas than in the slight fluorosis and non-fluorosis areas. No children with IQs of 120-129 and >129 were found in the medium and severe fluorosis areas.

In Table 4 the IQs of children of different ages from areas with differing prevalences of fluorosis are shown.

A comparison of the IQs of boys and girls is shown in Table 5.

Discussion

The results of the survey show that the intelligence, as measured by the mean IQ, of children aged 8-13 years living in areas with a medium or severe prevalence of fluorosis was lower than that of children living in areas with a slight prevalence of fluorosis or no fluorosis. Similarly more children with a low or borderline IQ were present in the medium and severe fluorosis areas. That a high fluoride environment can adversely affect the development of intelligence in children is in agreement with the findings of Guo *et al.*[1] No significant difference in intelligence was present between children in the slight fluorosis and the non-fluorosis areas. The lowering of intelligence in the moderate and severe fluorosis areas indicates that the central nervous systems of the children in those areas are adversely affected by fluoride.

Because no correlation was found between age and IQ for children in the medium and severe fluorosis areas, it appears that the influence of a high fluoride environment on the development of intelligence may occur early in development such as during the stages of embryonic life or infancy when the differentiation and

Table 2. Numbers of children examined and their mean IQs in areas differing in the prevalence of fluorosis

	Non-fluorosis area	Slight fluorosis area	Medium fluorosis area	Severe fluorosis area
Number of children examined	226	227	224	230
IQ (mean±SD)	89.9±10.4*	89.7±12.7	79.7±12.7**	80.3±12.9***

* comparing the non-fluorosis and slight fluorosis areas $t = 0.110$, $p > 0.05$.
** comparing the medium and severe fluorosis areas $t = 0.367$, $p > 0.05$.
*** comparing the severe and medium fluorosis areas, and the slight and non-fluorosis areas $t = 5.922$, $p < 0.01$.

Table 3. The distribution of child IQ scores from areas of differing fluorosis prevalence

Fluorosis status of area	IQ <70	IQ 70-79	IQ 80-89	IQ 90-109	IQ 110-119	IQ 120-129	IQ >129
Non	2.6%	9.7%	37.1%	46.8%	3.9%	0.8%	0
Slight	3.1%	15.9%	29.1%	47.1%	3.1%	1.3%	<0.4
Medium	25.4%	23.7%	29.9%	20.5%	0.4%	0	0
Severe	20.9%	26.6%	26.9%	25.2%	0.4%	0	0

Table 4. IQs of children of different ages from areas differing in the prevalence of fluorosis

	IQ (mean±SD) of children from areas in which the fluorosis prevalence was:			
Age in years[b]	non-fluorosis[c]	slight	medium[d]	severe
8.0 - 8.49	86.1±11.4	90.7 ± 9.7	78.9±13.9	83.8±14.4
8.5 - 8.99	88.9±13.9	87.2±20.2	79.8±10.0	80.2±15.1
9.0 - 9.49[a]	91.1±11.2	92.6±11.1	78.7±14.6	82.1±14.1
9.5 - 9.99	86.3±11.3	88.7±12.6	75.8±15.6	81.9±15.2
10.0-10.49[a]	88.4 ± 8.6	92.0±13.9	81.1±13.5	82.9 ± 9.0
10.5-10.99[a]	90.6 ± 9.1	91.7±12.6	75.5 ± 9.2	79.6±10.5
11.0-11.49[a]	92.2±13.1	86.7 ± 9.8	77.3±11.8	75.9±12.3
11.5-11.99[a]	91.7 ± 9.5	90.1±13.4	78.4±13.5	79.6±13.5
12.0-12.49	87.4 ± 5.9	86.8±10.4	85.9±12.8	76.9±14.3
12.5-12.99[a]	93.2 ± 5.7	86.6±10.2	85.5 ± 9.5	77.1±12.6
13.0-13.49[a]	93.8 ± 9.5	94.3±12.7	85.5±10.6	83.1±10.4

[a] comparing non-fluorosis and slight fluorosis areas, and medium and severe fluorosis areas $t = 1.945$-4.81, $p < 0.05$. For other age groups comparisons were not significant.
[b] comparing the IQ for one age group with that for other age groups in the same area, no significant differences were found $t = 0.03$-1.70, $p > 0.05$. No trend was found indicating that the IQ became lower as the time spent in a high fluorosis area increased.
[c] comparing non-fluorosis and slight fluorosis areas, no significant difference was found.
[d] comparing the medium and severe fluorosis areas, no significant difference was found.

Table 5. The IQs of boys and girls aged 8-13 from areas of differing fluorosis prevalence

	IQ (mean±SD) of children living in areas in which the prevalence of fluorosis was:			
	non-fluorosis	slight	medium	severe
Boys *	89.3±0.82 (n=148)	89.8±1.10 (n=139)	81.2±1.07 (n=132)	80.6±1.06 (n=151)
Girls	91.0±1.26 (n=78)	89.4±1.30 (n=88)	77.6±1.33 (n=92)	79.8±1.44 (n=79)

*comparing boys and girls in each of the areas differing in fluorosis prevalence p>0.5 (Standards established for the test show no significant difference between the sexes[4])

growth of the nervous system is most rapid. A higher concentration of fluoride has been found in embryonic brain tissue obtained from termination of pregnancy operations in areas where fluorosis due to coal burning was prevalent.[5-6] Stereological and ultramicroscopic study of this tissue showed the differentiation of brain nerve cells was poor, and brain development was delayed. This suggests that developing brain tissues are sensitive to the toxic effects of fluoride.

The findings in the present study suggest that in areas with a medium or severe prevalence of fluorosis, active and comprehensive measures should be taken to reduce the fluoride intake for the population, especially in pregnant women and infants. Avoiding a high intake of fluoride is seen to be a important factor in determining the quality of health enjoyed by communities.

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CHANGES OF THE HUMAN ERYTHROCYTE MEMBRANE PROTEIN SH BINDING SITE PROPERTY WITH EXPOSURE TO FLUORIDE AND THREE STRONG MUTAGENS

Y Y Wang, X J Li and W J Xin
Beijing, China

SUMMARY: The effects of three strong mutagens (potassium bichromate, mitomycin C, and colchicine) and fluoride on the human erythrocyte membrane protein SH binding site property have been studied by using the maleimide nitroxide-ESR technique. The results indicate that in singular and combined treatments with mutagens, the ratio of weakly to strongly immobilized components is reduced, so that the conformation of the weakly immobilized component protein is altered. It is possible that the inhibition in the cytogenetic response is induced by the interaction of fluoride with the other chemicals. There is a dose and temperature dependence of both the singular and the combined action of the mutagen on the membrane protein.

Key Words: Dose; Erythrocyte; Fluoride mutagenicity; Human; Membrane protein SH binding site property; Strong mutagens; Temperature.

Introduction

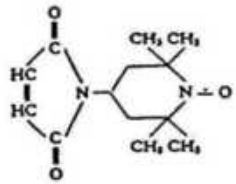
Fluoride (F^-) is a weak mutagen but inhibits a cytogenetic response induced by strong mutagens such as potassium bichromate (Cr^{6+}), mitomycin C (Mit C), and colchicine (Colch) by affecting sister chromatid exchange, micronuclei and cell cycle.[1-5] The inhibition of the cytogenetic response in the interaction of F^- with Cr^{6+}, Mit C and Colch has been postulated to be in relation to the conformational changes of membrane lipids and proteins involved in transport processes and permeability of the cell membrane.[6] Therefore, a better understanding of the behavior of fluoride on the cell membrane might help clarify the inhibition due to the interaction of F^- and other mutagens. Spin label electron spin resonance (ESR) technique is a valuable tool for studying the basic characteristics of biomembrane preparations, including conformation, permeability and fluidity.[7-9] Maleimide nitroxide used as a spin label can reveal more changes of the physical characteristics of cell membrane than stearic acid[10-11] and the ratio of weakly to strongly immobilized components (W/S) of the ESR spectra is very sensitive to the conformational changes of protein.[11] There is evidence of a close relationship between membrane protein SH groups and permeability.[12] The study of the inhibition by F^- of the effects of Cr^{6+}, Mit C and Colch on the membrane is based on earlier measurements of the latter three chemicals performed by using maleimide nitroxide-ESR technique. In the present study a new area of F^- research is investigated: the inhibition of the cytotoxic response induced by Cr^{6+}, Mit C and Colch, through interfering with the membrane protein SH binding site properties.

Beijing Municipal Research Institute of Environmental Protection. Fu Wai Avenue. Beijing 100037, China.

Materials and Methods

Erythrocytes: Healthy human erythrocyte suspensions (ACD-B), containing $1x10^9$ cells/ml, were supplied from the blood centre of the Red Cross Society in Beijing.

Spin labels: 3-Maleimide-PROXYL I was purchased from Aldich Co., USA. $7.5x10^{-4}$ mM maleimide solutions were prepared with isotonic Tris buffer.



Mutagens: Sodium fluoride and potassium bichromate AR were produced by BHC China. Mitomycin C injection was purchased from Kyowa, Japan. Colchicine was obtained from Fluk Co., USA.

Procedure: Dilutions of an erythrocyte suspension containing $1x10^7$ cells/ml were prepared in Tris-isotonic solution (1:100). The experiment was divided into two parts. In the singly treated group, Cr^{6+}, Mit C and Colch were singly added to the diluted erythrocyte suspensions. In the combined treated group, F^- with Cr^{6+}, Mit C and Colch respectively was added to the erythrocyte dilutions. A control group without adding any chemicals was set. After shaking and incubating for 15 h at 37°C, the mutagen-exposed erythrocyte solutions were centrifuged at 1000 g for 8.0 min. The supernatants were then added to maleimide spin label solutions (final concentration was $1.5x10^{-4}$ M) to label the mutagen-treated red cells. All the samples were incubated for 2.0 h at 37°C and subsequently centrifuged at 1000 g. The residual cells were rinsed three times with isotonic solutions to make the supernatants free from any signals of free spin labels, so that the ESR spectra would come only from the spin labelled erythrocytes. The spin-labelled cells were then put into a quartz capillary for ESR measurement, as previously described.[10-11]

Results and Discussion

Figure 1. A-B shows the representative ESR spectra obtained from human intact erythrocyte membrane, labelled with maleimide nitroxide I, after the mutagen exposure. Analysis of these spectra indicated that 2A values calculated from ESR spectra are identical: 66.7 ± 1.85G (mean ± standard error, SE) with a slight fluctuation of their peak amplitudes due to mutagen presence. The alterations of the signal amplitude are the result of both weakly and strongly reacting components, namely, the singular and combined treatments. It is possible that after exposure of cell membranes to any mutagen, the motions of the maleimide probes attached to immobilized binding sites do not encounter restrictions, and these probes do not move close enough to each other to result in dipolar broadening. It has been proven that the conformational changes of the membrane proteins could not be induced by exposure to the mutagen.

As shown in Table 1, the W/S value from human erythrocyte membrane exposed to Cr^{6+}, Mit C and Colch and labelled by maleimide is basically less than that of the untreated control group. The W/S ratio of erythrocyte membrane treated

by Cr^{6+} and Mit C decreases with the increasing dose. However, the W/S ratio with Colch exposure decreases at low concentration, but increases at high concentration, the increase starting at dose 7.5 μM.

When these results were compared to the effect of F^- alone,[10] the greatest reduction of the W/S ratio of the human erythrocyte membrane was found with Cr^{6+} and the least with Colch.

Figure 1. ESR spectra of the human erythrocyte membrane before and after the singular and combined treatment.

A. singular treatment:
a is control, b, c, d are Cr^{6+}, Mit C and Colch respectively.

B. combined treatment with mutagens:
a is control, b, c, d are F^-–Cr^{6+}, F^-–Mit C, F^-–Colch, respectively.

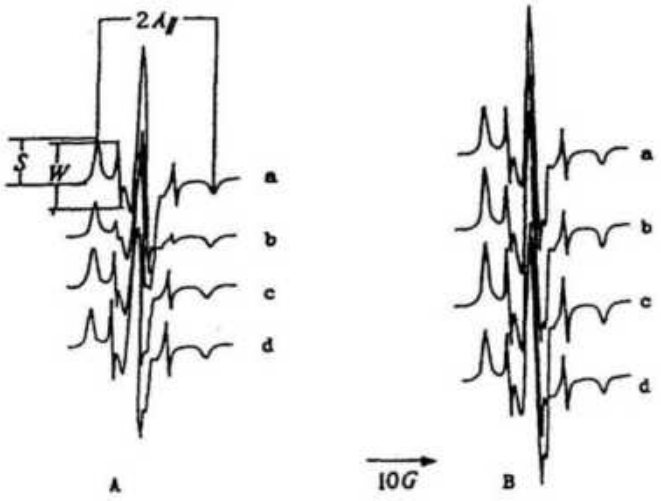


Table 1. W/S ratio of the human erythrocyte membrane before and after the treatment with various doses of mutagens

Dose	Cr^{6+}		Dose	Mit C		Dose	Colch	
μM	Mean	± SE	μM	Mean	± SE	μM	Mean	± SE
0	1.986	0.094	0	1.986	0.094	0	1.986	0.094
0.15	1.266	0.118	0.6	1.408	0.031	5.0	1.585	0.112
0.30	0.956	0.073	1.2	1.292	0.180	7.5	1.593	0.086
0.60	0.651	0.031	1.8	1.205	0.162	10.0	1.651	0.096
0.90	0.532	0.027	2.4	1.149	0.180	12.5	1.729	0.126

The results in Table 2 show that the W'/S' ratio, with the combined exposure of F^- with Cr^{6+} Mit C and Colch, decreases with increasing F^- dose. That is, Δ W'/S', the difference between W'/S' ratio of F^- and Cr^{6+}, Mit C and Colch, increases with the F^- dose (Figure 2).

W"/S" ratio in Table 3 decreases with the increasing dose of Cr^{6+} and Mit C with the combined exposure of a given dose of F^- (37.5 μM). Specifically, the Δ W"/S" ratio, the difference between W"/S" of F^- and Cr^{6+} or Mit C, increases with the dose of Cr^{6+} or Mit C respectively. The comparison shows that the difference between the inhibitory strength of Cr^{6+} or Mit C due to F^- is the same as the results in Table 2. However, W"/S' ratio of Colch decreases in low concentration and increases with higher concentrations when combined with a 37.5 μM F^- exposure. Thus, W"/S" ratio exceeds W/S value of 37.5 μM F^- exposure at the time Colch dose reaches 12.5μM, exhibiting the same response

Table 2. W'/S' ratio when the various doses of F^- were combined with Cr^{6+}, Mit C or Colch

Group		F^-(μM)				
		0	5.0	12.5	25.0	37.5
Cr^{6+}	Mean	0.532	0.530	0.479	0.477	0.467
(0.9 μM)	± SE	0.027	0.069	0.054	0.050	0.045
Mit C	Mean	1.149	0.930	0.866	0.820	0.764
(0.4 μM)	± SE	0.180	0.042	0.047	0.040	0.018
Colch	Mean	1.585	1.358	1.319	1.205	1.167
(5.0 μm)	± SE	0.112	0.005	0.051	0.021	0.035

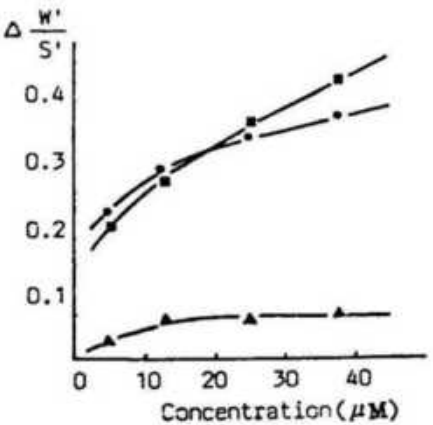


Figure 2. The dose curves in W'/S' of sodium fluoride

▲ Cr^{6+} ● Mit C ■ Colch

W'/S' : W/S ratio obtained from combined treatment with a certain dose of Cr^{6+}, Mit C or Colch with various doses of F^-.

Δ W'/S' : The difference between W'/S' of F^- and W'/S' of Cr^{6+}, Mit C or Colch.

as Colch singularly treated erythrocyte membrane. As a result Δ W"/S" ratio, the difference between W"/S" ratio of F^- and Colch exposure, becomes negative. That is, the point of Δ W"/S" lies in the fourth quadrant in Figure 3.

Analysis of the above data also suggests that there is a parallel relationship between the membrane protein SH binding site property and the dose of mutagens in the singular and combined treatments.

The results of reductions in the W"/S" ratio in singular treatment, with F^-, Cr^{6+} and Mit C may occur through the same mechanism with the oxidation of protein SH groups on the human erythrocyte membrane to S-S linkages. As is well known, Cr^{6+} is a very strong oxidant.[13]

Table 3. W"/S" ratio when the various doses of Cr^{6+} Mit C or Colch were combined with 37.5 μM F^-

	Dose (μM)	0	0.15	0.30	0.60	0.90
Cr^{6+}	Mean	1.360	0.990	0.852	0.619	0.542
	± SE	0.065	0.023	0.026	0.026	0.014
	Dose (μM)	0	0.6	1.2	1.8	2.4
Mit C	Mean	1.360	1.069	0.982	0.944	0.895
	± SE	0.065	0.095	0.112	0.103	0.127
	Dose (μM)	0	5.0	7.5	10.0	12.5
Colch	Mean	1.360	1.281	1.311	1.334	1.373
	± SE	0.065	0.111	0.059	0.062	0.063

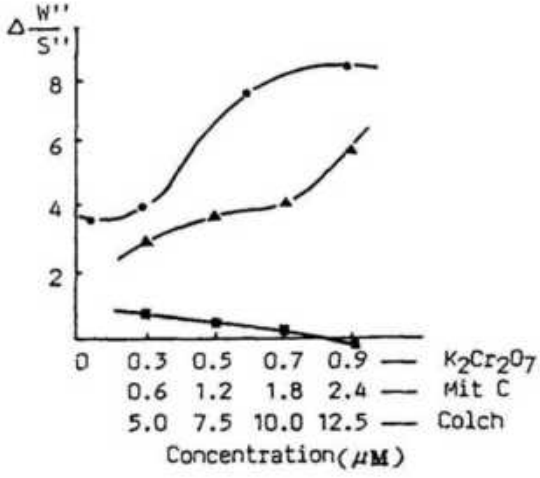


Figure 3. The dose curve in W"/S" of Cr^{6+}, Mit C or Colch

Δ Cr^{6+} ● Mit C ■ Colch

W"/S": W/S ratio obtained from combined treatment with 37.5 μM F^- respectively with various doses of Cr^{6+}, Mit C or Colch.

Δ W"/S": The difference between W"/S" of F^- and W"/S" of Cr^{6+}, Mit C or Colch.

Mit C contains a quinone group and can release an active oxygen free radical,[14] and F^- is strongly electronegative.[15] Besides, analysis of ESR spectra reveals that the reductions of W/S ratio are not the results of damage to cell membranes (constant W while S value changes) but rather arise mainly from a decrease of the weakly immobilized component. The oxidations by the three mutagens of the SH-groups of the weakly immobilized component of the protein may be explained by the actions of the chemicals on the membrane surface only. That is, the contribution to the SH/S-S rate in the membrane depth is higher than that in the surface. In consequence, the conformational changes of membrane protein are induced by mutagens.

Colchicine is a mitotic poison distinguished from the other three mutagens as a chromosomal clastogen. Although Colch can cause the changes of the association and functioning of microtubules and microfilaments due to non-covalent binding with microtubule protein, its chemical properties are not yet fully understood.[16] Colch appears to decrease the weakly immobilized component protein SH groups on erythrocyte membrane. The phenomenon whereby the W/S ratio is decreased and then increased in the course of Colch exposure, as seen in Figures 2 and 3, is not understood at present.

The results in Table 4 show that the W/S ratio of erythrocyte membrane, singly treated by Cr^{6+}, Mit C and Colch and in the control, increases with the temperature between 0 and 50°C. A point of phase transition, in the course of the temperature changes, exists in each measurement group: 28.5°C in the control, 28.8°C in Cr^{6+}, 25.5°C in Mit C, 24.0°C in Colch, and 22.5°C in F^- [10] (Figure 4).

The results in Table 5 indicate that the W/S ratio of erythrocyte membrane jointly treated by F^- with Cr^{6+}, Mit C and Colch increases with the temperature between 0 and 50°C. A point of phase transition exists in each group: 24.0°C in F^--Cr^{6+}, 25.0°C in F^--Mit C and 27.5°C in F^--Colch (Figure 5).

There is a temperature dependence of the W/S ratio in cell membrane treated by Cr^{6+}, Mit C, Colch and F^- singly, and by F^- with the former three mutagens jointly. A transition temperature exists with each group. Compared with the control, the phase transition point in erythrocyte membrane exposed to F^-, Mit C and Colch is decreased 6.0°C, 3.0°C, and 4.5°C, but with Cr^{6+} is increased 0.3°C, respectively, while with F^--Cr^{6+}, F^--Mit C and F^--Colch the transition point is reduced 4.5°C, 3.5°C and 1.0°C, respectively. As compared with the corresponding mutagen, there is 4.8°C and 0.5°C decrease of Cr^{6+} and Mit C but 3.5°C increase of Colch.

The alteration of the phase transition point caused by F^- of 6.0°C was greater than that caused by the other mutagens Mit C, Colch and Cr^{6+}. This fact suggests that F^- has a stronger effect on the membrane protein SH binding sites than the other three mutagens. When F^- was combined with the other three mutagens the difference between the phase transition points was not enhanced as much as with F^- alone.

Temperature appears to affect the membrane protein structure so that the W/S ratio increases with the temperature, inducing change in the membrane conformation. The result is that the W/S ratio of the treated group is less than that of the control, because the more the protein SH group of the weakly immobilized binding site is exposed with temperature change, the more the SH group is affected by the mutagen.

Table 4. Change of W/S ratio with temperature in singular treatment with mutagens

Group	Temperature	0°C	10°C	20°C	25°C	30°C	35°C	40°C	50°C
Control (0.0μM)	Mean W/S	0.697	0.918	1.136	1.257	1.356	1.520	1.639	1.944
	± SE	0.012	0.010	0.014	0.008	0.014	0.026	0.024	0.067
Cr^{6+} (0.9Mμ)	Mean W/S	0.301	0.387	0.470	0.523	0.585	0.639	0.725	0.917
	± SE	0.031	0.031	0.039	0.044	0.056	0.064	0.068	0.095
Mit C (2.4μM)	Mean W/S	0.475	0.639	0.762	0.826	0.921	1.003	1.100	1.318
	± SE	0.013	0.025	0.024	0.021	0.012	0.018	0.011	0.014
Colch (5.0μM)	Mean W/S	0.689	0.902	1.128	1.230	1.363	1.501	1.630	1.907
	± SE	0.017	0.022	0.005	0.012	0.033	0.031	1.027	0.042

Figure 4 (below left) Temperature curve of human erythrocyte membrane before and after singular treatment with mutagens.

o Control ▲ Cr^{6+}
● Mit C ■ Colch

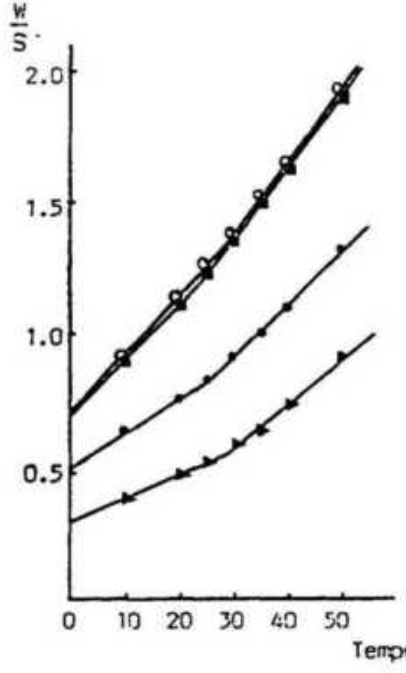


Figure 5 (below right) Temperature curve of human erythrocyte membrane before and after combined treatment with mutagens.

▲ Cr^{6+} ● Mit C ■ Colch

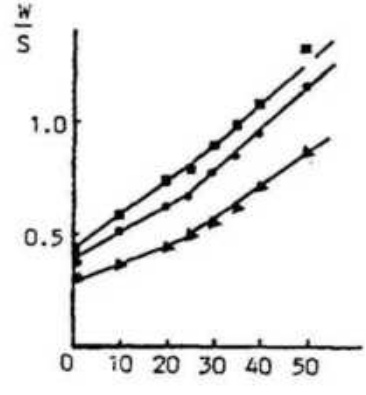


Table 5. Change of W/S ratio with temperature in combined treatment with mutagens

Group	Temperature	0°C	10°C	20°C	25°C	30°C	35°C	40°C	50°C
F^--Cr^{6+} (37.5-0.9μM)	Mean W/S	0.283	0.364	0.448	0.500	0.556	0.619	0.703	0.876
	± SE	0.017	0.023	0.027	0.028	0.029	0.042	0.046	0.041
F^--Mit C (37.5-2.4μM)	Mean W/S	0.429	0.584	0.734	0.779	0.889	0.991	1.069	1.330
	± SE	0.062	0.072	0.106	0.110	0.118	0.145	0.131	0.178
F^--Colch (37.5-5.0μM)	Mean W/S	0.614	0.761	0.954	1.040	1.161	1.259	1.453	1.634
	± SE	0.020	0.019	0.018	0.020	0.015	0.020	0.042	0.058

Conclusion

The results of the present study coupled with our previous work demonstrate that the fluidity and protein SH binding site property in human erythrocyte membrane are changed by the four mutagens fluoride, potassium bichromate, mitomycin C and colchicine. The conformations of the membrane lipids and proteins are also changed with resulting alterations in membrane function including permeability. When fluoride interacts with the other three mutagens the changes are inhibited. This inhibition results in a molecular response, possibly through the transmembrane protein of the erythrocyte membrane Band 3, which mediates anion exchange across the red cell membrane. Further exploration of the action of fluoride on the cell membrane may help ways to be found for protection against fluoride toxicity.

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HIGH FLUORIDE CONTENT OF FOOD AND ENDEMIC FLUOROSIS

Y Z Han, J Q Zhang, X Y Liu,[a] L Z Zhang,[b] X H Yu[b] and J A Dai
Hefei, China

SUMMARY: A survey was carried out in a village of an endemic fluorosis area. There was a high rate of dental fluorosis (80.4%). Children 8-15 years old had the highest rate (94.7%). Fluoride content of drinking water, from two wells, was low (0.14 and 0.70 mg/L). Samples of food and tea were also tested. Total daily fluoride intake per adult was 4.14 mg. Fluoride in food and tea was 70% of the total fluoride intake, and therefore was the main factor causing the endemic fluorosis.

Key words: Endemic fluorosis; Fluoride intake; Food; Tea.

Introduction

In an epidemiological investigation of endemic fluorosis in Jixi county of Anhui province, 45 endemic villages were discovered. The fluoride content of drinking water was less than 1.0 mg/L in 44 villages and under 0.5 mg/L in 33 villages. There were high rates of endemic fluorosis. The rate for children between 8-15 years was 100% in some villages. Because the source of the fluoride was unclear, this study was done in Tongkang village of Jixi county in 1988.

Materials and Methods

Tongkang village was selected randomly and a survey for dental and skeletal fluorosis was conducted on all 158 residents, of whom 148 (93.7%) were examined. Diagnostic criteria were based on "prevention and treatment standards for endemic fluorosis" of the Ministry of Public Health, China.[1]

Drinking water and food samples were collected and the fluoride content was determined by the fluoride ion selective method.[1,2]

Total fluoride intake from water, foods and tea was calculated.[3]

Results

Tongkang village is located in a mountainous region with sandy soil and abundant precipitation. Residents are engaged in agriculture throughout the year. Their main food is rice, along with sweet potatoes and vegetables as additional staples. Their common beverage is tea, often steeped in a thermos.

Fluorosis prevalence: See Table 1. The mean dental fluorosis prevalence rate was 80.4%. Percentages of chalky, discolored, and pitted teeth were 15.1%, 62.2%, and 22.7%, respectively. The dental fluorosis prevalence rate among children aged 8-15 years was 94.7%. No cases of skeletal fluorosis were found.

Fluoride content of food samples is shown in Table 2. Most kinds of tea were of poor quality. Intake from other foods was: main food (rice) 0.6 kg, subsidiary foods 0.3 kg, vegetables 0.7 kg, salt 20 g, tea 5 g.

Anhui Anti-epidemic Station, 125 Wuhu Road, Hefei, Anhui 230061, People's Republic of China. [a] Huang Anti-epidemic Station. [b] Jixi County Anti-epidemic Station.
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Fluoride content of water from the two wells used was 0.14 and 0.70 mg/L. Average drinking water intake by adults was 2.5 L (including 1.5 L tea water).

Calculated total fluoride intake from water, food, and tea averaged 1.05 mg, 2.30 mg and 0.78 mg per adult per day, respectively, making a total average intake of 4.14 mg per adult per day. Foods and tea, accounting for more than 70% of the total fluoride intake, were the main sources of fluoride in Tongkang village.

TABLE 1. Prevalence rates of fluorosis in village residents

	n	Percent	
Population of village	158		
Survey total	148	93.7%	of 158 residents
Dental fluorosis prevalence	119	80.4%	of 148 surveyed
with chalky white teeth	18	15.1%	of 119 with fluorosis
with discolored teeth	74	62.2%	of 119 " "
with pitted teeth	27	22.7%	of 119 " "
Children 8-15 years	18	94.7%	of 19 aged 8-15 yr

TABLE 2. Fluoride content of food samples

Type	No. of samples	Mean mg/kg ± SD	Range mg/kg
rice	10	0.85 ± 0.20	0.40-1.08
sweet potato (dry weight)	10	1.09 ± 0.32	0.69-1.60
soybean	16	0.62 ± 0.09	0.52-0.74
vegetable (dry weight)	16	2.76 ± 0.80	1.60-4.60
radish (dry weight)	6	1.57 ± 0.52	0.60-2.60
salt	6	1.08 ± 0.49	0.52-2.00
tea	8	152.94 ± 75.32	90.00-300.00

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ENDEMIC FLUOROSIS IN SAN LUIS POTOSI, MEXICO. II. IDENTIFICATION OF RISK FACTORS ASSOCIATED WITH OCCUPATIONAL EXPOSURE TO FLUORIDE

Jaqueline Calderón,[a] Isabelle Romieu,[b] Magdalena Grimaldo,[a]
Héctor Hernández[c] and Fernando Díaz-Barriga[a]
San Luis Potosi, Mexico

SUMMARY: The city of San Luis Potosi (SLP), Mexico, is located in an area where drinking water contains excessive quantities of natural fluoride. Also in this city is located a small industry that produces hydrofluoric acid. In order to investigate both routes of exposure to fluoride (industrial air and drinking water), we conducted a pilot study in workers of this industry. The study involved 60 male workers, divided into two groups according to their work area: the production and the office groups. Although the exposure to fluoride by the water ingestion pathway was similar for both groups, the occupational exposure to fluoride was 12 times higher in the production area. Workers in this area had higher levels of fluoride in urine than workers in the office area. This difference was observed in the preshift and the postshift samples. A multivariate regression analysis showed that the workplace explained 33% of the fluoride content of the urinary samples, whereas tap water ingestion explained only 8%. The higher air fluoride levels in the production area could explain the high number of workers who present a preclinical phase of skeletal fluorosis. Although our results illustrate the exposure to fluoride of workers in the production area by two pathways, water and workplace air, it would be advisable to explore in more detail the participation of other pathways of exposure, like diet and soft drinks.

Key words: Air fluoride; Drinking water fluoride; Endemic fluorosis; Occupational fluorosis; Skeletal fluorosis.

Introduction

Skeletal fluorosis is the principal health problem associated with occupational exposure to fluoride.[1,2] The most important risk factor in determining whether skeletal fluorosis will occur and how severe it will be, is the total amount of fluoride consumed from all sources.[1,2] At the occupational level, the principal source of fluoride is industrial air, whereas in endemic areas with naturally fluoridated drinking water, the ingestion of contaminated water is the main source of fluoride.[1,2] Therefore, the highest risk would be observed in people that simultaneously are occupational exposed to fluoride and reside in endemic areas with higher than normal levels of fluoride in drinking water.

The city of San Luis Potosi (SLP), Mexico, is located in an area where drinking water contains excessive quantities of natural fluoride.[3] Also, in this city, is located a small industry that produces hydrofluoric acid. In order to investigate both pathways of exposure to fluoride (industrial air and drinking water), we conducted a pilot study in workers of this industry.

[a]Facultad de Medicina, Universidad Autónoma de San Luis Potosí. [b]Centro Panamericano de Ecología Humana y Salud (OPS/OMS). [c]Centro Médico del Potosí.
Address all correspondence to: Dr. Fernando Díaz-Barriga, Facultad de Medicina, Avenida Venustiano Carranza No. 2405, 78210, San Luis Potosí, SLP, Mexico. Phone (52-48) 130 499. Fax (52-48) 176 976.

Methods

Study Population

The study, which was conducted in 1994, included 60 male workers selected at random, divided into two groups according to their work area. Two areas were studied, the production area and the office area. The former has been identified as a high risk area because of the exposure to fluoride during the production of hydrofluoric acid, whereas the office was recognized as a low risk area. The workers in the office area were between 24 and 66 years old, had worked at the industry for 1 to 20 years, and had a history of residence in the city of San Luis Potosí of 22.2 ± 18.0 years. The workers in the production area were between 21 and 47 years old, had worked at the industry for 2 to 26 years, and had a history of residence in the city of 22.3 ± 10.6 years. The differences among groups were not statistically significant. An occupational questionnaire was administered to all the workers by the same interviewers.

Skeletal Fluorosis

Prevalence of skeletal fluorosis was determined according to the classification of the DHHS.[1] Preclinical phase was recorded when slight increase in bone mass was detected; whereas clinical phase I was associated with osteosclerosis of the pelvis. Therefore, radiographs of the pelvis (antero-posterior view) were obtained from the workers. The technique of radiographic examinations was standardized as much as possible. An independent observer, without knowledge of the work history of the individual, read all the radiographs. As a quality control for the radiograph interpretation, four workers were also studied by densitometry (vertebral column and region of the hip). The densitometries were read by a second independent observer, also without knowledge of the work history of the individual.

Urinary Fluoride Analysis

NIOSH method, "fluoride in urine", was followed.[4] As an internal quality control program, primary standard reference material was analyzed (QA/QC samples of fluoride in urine from WHO/HEAL/TCC in China). Our fluoride recovery was 104%. Fluoride was measured in preshift and postshift samples.

Drinking Water Samples

Tap water samples were collected from the homes of the 60 workers included in the study. Samples were collected in polyethylene bottles. Fluoride was quantified within 24 h from sampling. Fluoride levels were quantified by adding TISAB buffer to the samples just prior the analysis with a sensitive specific ion electrode. As an internal quality control program primary standard reference material was analyzed (QA/QC samples of fluoride in water from WHO/HEAL/TCC in China). Our fluoride recovery was 106%.

Industrial Air Samples

The method of Bonney and Farrah[5] was followed. Gaseous fluoride was measured with personal sampling pumps. Samples were obtained for an 8.0 hour period at least monthly during the last seven years. Fluoride levels were quantified in the aqueous solution where the fluoride from the alkaline impregnated cellulose pad was transferred. Measurements were done with a sensitive specific ion electrode. Standards for gaseous fluoride were used.[5]

Statistical Analysis

The distribution of urinary fluoride levels was skewed. Therefore values were log-transformed. Fluoride levels in urine between groups of workers were compared by t test. Within groups, the differences between preshift and postshift samples were analyzed with paired t test; one-tailed test was used with a significance level of α=0·05.[6] Simple linear regression models were used to defined significant predictors of fluoride in urine. These predictors were then included in a multivariate regression model.[6] The analyses were carried out with the SPSS-PC statistical package.

Results and Discussion

Exposure to Fluoride

We obtained samples of drinking water from the workers' residences and no differences were observed in the fluoride content between the groups. For the office group, fluoride levels in samples of tap water collected at the workers' residences (n = 24) had a mean of 2.29 mg/L (range 0.67 - 3.75 mg/L), whereas for the production group, the samples collected at the workers' residences (n = 36) had a fluoride mean level of 2.22 mg/L (range 0.54 - 4.36). At the plant, the only source of potable water had a fluoride level of 0.69 mg/L. With these results, and considering that the differences in water consumption between office and production workers were not significant; it can be established that the exposure to fluoride by water ingestion was similar for both groups. In contrast, the occupational exposure to fluoride was 12 times higher in the production area than in the office area (Table 1). The fluoride mean concentration in air at the production area for the period 1987-1988, was close to the recommended NIOSH's exposure limit of 2.5 mg/m^3.[2] When compared, air fluoride levels in the production area were lower during the period 1990-1994 than during the period 1987-1988 (Table 1). However, some samples (Table 1) continued to be above average (air levels in the upper limit of the range).

TABLE 1. Fluoride concentration in workplace air (mg/m^3)

AREA	n	YEAR	MEAN	S.E.	RANGE
Office	3	94	0.017	0.005	0.01 - 0.02
Production	25	87-88	1.78	0.30	0.07 - 4.54
Production	57	90-94	0.21	0.07	0.02 - 2.02

Eight hours samples. Results are given as geometric mean. n = number of samples. S.E. = standard error. The office area was monitored only during the study period.

Table 2. Preshift and postshift urinary concentrations of fluoride (mg/L)

	AREA	n	G. MEAN	S.E.	RANGE
PRESHIFT	office	24	1.79	1.09	0.7 - 4.6
	production	36	3.98	1.13	0.7 -15.9
POSTSHIFT	office	24	2.25	1.10	0.8 - 4.9
	production	36	5.12	1.09	1.4 -18.9

The mean differences were statistically significant. Between areas $p < 0.001$. Between shifts: in office area $p < 0.03$; in production area $p < 0.01$.

TABLE 3. Pearson correlations of fluoride in urine and different continuous variables

VARIABLE	r	p
fluoride content in water (mg/L)	0.31	0.007
age (years) *	- 0.24	0.03
time working in industry (years) *	0.10	0.22
time of residence (years) *	- 0.21	0.08

* These variables were log transformed for the analyses

TABLE 4. Predictors of fluoride in urine

VARIABLE	ß	S.E.	p
Tap water ingestion	0.08	0.02	0.002
Workplace	0.33	0.05	0.001

R^2 = 0.45. ß is the estimated coefficient of the variable in the regression, S.E. is the standard error and p is the significance. Water ingestion in mg/L. Workplace refers to office or production areas.

TABLE 5. Skeletal fluorosis prevalence among workers

AREA	n	Normal	Preclinical phase	Skeletal fluorosis I	Others
Office	20 (100%)	12 (60%)	3 (15%)	1 (5%)	4 (20%)
Production	35 (100%)	16 (46%)	13 (37%)	2 (6%)	4 (11%)

Others refer to lesions not related to skeletal fluorosis . Diagnoses were done by radiograph analysis. Four workers in the office area and one worker in the production area were lost for this part of the study. The differences were not statistically significant.

TABLE 6. Densitometry of the vertebral column and of the hip

AREA	Normal	Preclinical phase	Skeletal fluorosis I	Skeletal fluorosis I
Vertebral Column L1-L4	105	103	129 *	114 *
Neck of the Femur	112 *	111 *	115 *	121 *
Ward Region of the Femur	103	102	106	137 *
Greater Trochanter of the Femur	99	110 *	118 *	123 *

Each column represent a worker with the diagnosis obtained by radiographs. Percentage of bone density was adjusted by age, weight and race. * Bone densities 10% above the normal reference value.

Urinary Fluoride Levels

Preshift samples were provided during the morning of the first day following the 48 hours rest period of each worker. Postshift samples were provided during the morning of the fifth day of the working period. Results are shown in Table 2. Workers of both areas had statistically significant higher urinary fluoride levels in the postshift sample. Furthermore, workers in the production area had higher levels of fluoride in urine than workers in the office area. This difference was observed in the preshift and the postshift samples. The higher fluoride levels in the urine of

workers belonging to the production area can be explained by a higher exposure to fluoride due to the levels of fluoride in air registered at this area (Table 1).

Interestingly, 72% of the workers in the production area had fluoride urinary levels above the preshift biological exposure index of 3.0 mg/L,[2] whereas only 8.3% of the workers in the office area had levels above this index. Whether this difference can be explained by a higher fluoride excretion, due to an increased body burden of fluoride in workers of the production area, is a matter that certainly needs further research. However, in agreement with this interpretation, it has been described that large amounts of fluoride were excreted for prolonged periods by persons who lived for many years in areas with high fluoride water levels and who subsequently moved to areas with low fluoride levels.[7] The postshift biological exposure index of 10.0 mg/L[2] was surpassed by 5.6% of the workers in the production area, but by none of the office's workers.

The correlation coefficient between different continuous variables and urinary fluoride levels showed significant results only with age and fluoride content in water (Table 3). Urinary fluoride and workplace areas also showed significant differences ($p < 0.001$). With the significant variables of the univariate analysis, a fitted multivariate regression model was obtained. In this model workplace explained 33% of the fluoride content of the urinary samples, whereas tap water ingestion explained only the 8% (Table 4). Together, the workplace and the tap water ingestion explained 45% of the total fluoride in urinary samples. In consequence, we have to take other sources into account in order to totally explain fluoride exposure. Among them, we had previously identified the following: boiled water; "soft drinks"; and food preparation with boiled water.[3]

Skeletal Fluorosis

In our study, skeletal fluorosis was defined according to the classification of the Department of Health and Human Services, USA.[1] Table 5 shows the distribution of skeletal fluorosis among the two workers' groups. The production workers had a higher although not significant prevalence of the preclinical phase of skeletal fluorosis than office workers. It is noteworthy that among the office workers, 20% ($n = 4$) had signs of skeletal fluorosis. This suggests that environmental exposure (excluding occupational exposure) would lead to severe impairment.

As a quality control for the radiograph-based diagnosis of skeletal fluorosis, four workers were also studied by densitometry (Table 6). The results obtained by densitometry were concordant with data obtained by radiographs (Table 5). Workers with a diagnosis of skeletal fluorosis (clinical phase I) had a higher bone density than workers without signs of skeletal fluorosis (normal).

Three of the four skeletal fluorosis cases in the office group were found among workers older than 45 years, whereas 73% of the cases in the production group were found in workers aged 35 years or more. The reason for the appearance of skeletal fluorosis in younger workers in the production area, could be the additional exposure to fluoride due to the presence of this mineral in workplace air (Table 1). A clear association between changes of early skeletal fluorosis and time of occupational exposure has been reported.[8] However, in our study, no correlation was found between the frequency of skeletal fluorosis and the time working in the industry. This could be explained by the fact that in our study workers were exposed to fluoride by two different pathways (water and workplace air), there-

fore, the changes associated to skeletal fluorosis are not due just to the occupational exposure.

In agreement with previously reported data,[8] there was no correlation between the presence of abnormal pelvic radiograph findings and the presence of musculoskeletal complaints. This could be easily explained, assuming that the prevalence of musculoskeletal complaints depend more on occupational activities than on the exposure to fluoride.

Conclusions

As expected, these results proved that workers in the production area are at risk of fluorosis because of their exposure to fluoride by two pathways: water and workplace air. However, considering that the workplace and the tap water ingestion explained only 45% of the total fluoride in urinary samples; it would be advisable to study other sources for fluoride exposure in this population, such as: boiled water; "soft drinks"; and food preparation with boiled water.[3] Furthermore, it is clear that the alteration in bone density, or modification of the trabecular structure, are nonspecific indicators of fluoride toxicity. Therefore, there is a need to find more specific biomarkers for skeletal fluorosis, especially for the preclinical phase. The biomarkers could be a major advance in detecting early skeletal fluorosis, and for developing health programs to prevent crippling fluorosis.

Acknowledgment

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In the October 1992 issue of *Fluoride*, we published tributes to the late John Marier, of the National Research Council of Canada. One tribute mentioned the award to John, in 1985, of a Certificate of Merit from the American Society for Magnesium Research. The other, commenting on John's lack of formal scientific training, recalled his "remarkable ability to read scientific papers, to retain details of what he had read, to mull them over in his mind seeking the inter-relations of items from different authors and to formulate therefrom a suggestion for further research or even a new scientific hypothesis." In light of the subject of the current issue's guest editorial, and the continuing debate on fluoridation, it seemed appropriate to publish the following piece, written 29 years ago. - Editor

THE QUESTION OF FLUORIDATION

the late J R Marier
Ottawa, Ontario, Canada

To many people, the above title might seem inappropriate, because many are of the opinion that fluoridation is beyond question. However, to one who has delved into the scientific aspects of the subject, fluoridation can remain as contentious and unresolved as ever before. This summary attempts to show, on the basis of scientific evidence, why the fluoridation topic should still remain a subject of much soul-searching, not only by scientists directly involved in fluoride research, but by all scientists and interested (and informed) laymen. The main difficulty in assessing the situation was touched upon recently by a world-famous researcher in a private letter to a fellow researcher: "I have carefully avoided getting into the controversy, which is a political and not a scientific one". During the past two decades, it has become fashionable to brand opponents of the fluoridation program as "cranks... sensation seekers... quacks... pseudo-scientists... misinformed... biased..." and other unflattering terms. Along with this, it has become customary to give widespread publicity only to those scientific reports which supports the fluoridation program. Adverse findings are available, but they are scarcely (if ever) mentioned in the wide-circulation news media. Whatever the merits or demerits of the fluoridation program, the strict avoidance of negative findings is not healthy to the cause of fluoridation, nor of Science itself, because Science depends for its survival on the unrestricted dissemination of *all* scientific findings in its quest for truth. So, for the truth to be known about fluoridation, it is essential that all sides of the question be given close scrutiny and, also, that a sense of objectivity should prevail. Fluoridation should not be a matter of "faith", but a matter of "fact".

Space does not permit the re-telling of the entire history of the fluoridation program. Such information is readily available in the numerous books devoted to the subject, most of which have championed the advantages of the scheme. Because this summary has as its specific goal the bringing to light of little-known information, let us merely remember that, 20 years ago, it was stated that no undesirable effects of fluoride would occur in regions using water containing less than 10 parts per million ("ppm") of fluoride. A few years later, this was lowered to 8 ppm. then 4 ppm. Recently, Dr Bertram Sauerbrunn and his colleagues at the Washington DC Veterans Administration Hospital have reported a fatality in

Texas. The man had advanced skeletal fluorosis, yet had never lived in a region where the fluoride content of the water was higher than 3.5 ppm (on a life time basis, the man had been exposed to an average concentration of only 2.5 ppm of fluoride in the water!). This revelation received no publicity. Instead, the public was bombarded with press-releases claiming that fluoride was "beneficial" to the bones of the aged, and the claim made that fluoride would eliminate osteoporosis (a rarefaction of bone which occurs in old age). Yet even *this* claim was incomplete, because no mention was made of the fact that, when fluoride is used to combat osteoporosis, the new bone formed is abnormal, *i.e.*, it is the same kind of pathological bone encountered in skeletal fluorosis! At least two diseases (or conditions) have been reported in which fluoride actually aggravates the condition: in pyelonephritis (a kidney ailment) and post-parathyroidectomy patients.

Yet, despite the availability of many such reports, the concentration of fluoride recommended for fluoridation programs (the sacrosanct "1.0 part-per-million") is deemed to be entirely safe. An examination of the scientific literature reveals that this is not the case. Dr M A Roshal, in a 1965 issue of the journal issued by the Leningrad Medical Institute, reported that intake of fluoride - even at the apparently "safe" concentration of 1.0 ppm - caused derangements in blood sugar balance. The implications of these findings as regards those afflicted with diabetes should become a concern. The effect of fluoride on people afflicted with the reverse condition of hypoglycæmia should also be borne in mind. And yet, this is another finding that received no publicity; nor is it the subject of any general follow-up research - even though a case-history was recently described in a 1966 issue of the *University of Ottawa Medical Journal*.

Another finding of interest is the recommendation that fluoridated water (at 1.0 ppm) should be *avoided* for hæmodialysis (*i.e.*, artificial kidney) treatments. This is another case that terminated with a human fatality, yet still another that went unpublicized. Convulsive seizures were one of the symptoms, along with an abnormally-high serum phosphorus level. The same symptoms had been described by Detroit's Dr G L Waldbott in a 1957 report dealing with an adverse effect of water containing 1.0 ppm of fluoride. Strangely enough, the later hæmodialysis report did not refer to the symptoms described by Waldbott. Here, we see a case where the failure to publicize adverse findings may well have hindered the fluoride researchers themselves. The recent increase in scientific output throughout the world has made it almost impossible for researchers to keep completely abreast of all developments in their field, unless *all* aspects of the field (even if they run counter to "established " beliefs) are given equal publicity in those periodicals devoted to scientific commentary. It was once deemed "heresy" to advocate that the earth travelled around the sun - yet we all know (or *should* know) what eventually happened.

There have been other revelations in connection with 1.0 ppm water. In a 1963 report, Dr H C Hodge of the University of Rochester described cortical defect in the long bones of children living in Newburgh NY (using artificially-fluoridated water containing 1.0 ppm of fluoride). Although, at the time, these bone changes

were considered to be "normal variants". Dr Hodge suggested that the cases deserved "additional study". No report dealing with such "additional study" has yet appeared.

In the mid to late 1950s, Dr Ional Rapaport of Wisconsin University published a series of articles in which he warned that the incidence of mongoloid births would increase in districts using fluoridated water. His reports were scarcely referred to by advocates of the fluoridation program and, when they were, they were downgraded by recourse to the uncomplimentary terminology cited at the outset of this article. No follow-up study was ever undertaken to fully assess the merits of Rapaport's conclusion, even though he had suggested that the cause of the mongoloid disorder was a (fluoride-induced) interference with the metabolism of tryptophan (an essential amino-acid). Now, in 1967, an article in the British medical publication *Lancet* reveals that the administration of 5-hydroxytryptophan to mongoloid infants greatly improved the muscle-tone in such infants. So, it would seem that Dr Rapaport was not altogether crazy, even though the vital connection with fluoride still remains to be elucidated.

In 1963, a group of Canadian scientists caused a furore by publishing a review article asking that more thorough research be done on the fluoride subject. They were concerned about the crucial roles of minerals such as calcium and magnesium, and of Vitamins such as D and C. They feared the effect of fluoride on the solubility of bone mineral (since confirmed), the increasing infusion of fluoride into all our food products (the result of the use of fluoridated water for processing), and recommended the fluoridation of milk rather than water. They pointed out that, in North America, most of the "natural" fluoridated waters are hard waters, containing considerable amounts of calcium and magnesium. Thus they questioned the wisdom of fluoridating soft waters. The Canadian group has authored two subsequent articles, showing that many of their contentions have since been borne out by other workers, and have themselves shown that food items processed with fluoridated water contain from 0.6 to 1.0 ppm of fluoride, instead of the "normal" 0.2 to 0.3 ppm. The presence of so much more fluoride in our present-day food products calls for a critical examination of the "baseline" on which the fluoridation program was founded 20 years ago, when the large metropolitan food and beverage processors were not using fluoridated water. The Canadian researchers had also stated that intake of fluoride by pregnant mothers was useless, in terms of conferring a dental benefit to the offspring. This has since been admitted by Schlesinger, who, however, questioned the economics of fluoridating milk, even though Switzerland's Dr Robert Wirz had shown that the dental benefits obtained with fluoridated milk "are comparable to those of water fluoridation *with the advantage of considerable less cost*".

In addition to the above reports, the Canadian researchers have been interested in the connection between magnesium deficiency and fluoride-induced ailments. There are many symptoms common to both conditions: unusual bone growth, soft-tissue calcification, muscular twitching, tetaniform convulsions with an increase in serum phosphorus level, *etc*. But, to date, no scientific study has been

undertaken to correlate the two syndromes, although a few preliminary trials have been revealed that the result depends on the type of animal tested (note: the same observation was made during the recent "cobalt in beer" trials, *after* the Quebec City fatalities).

Another condition deserves mention. Workers in Russia, India, Yugoslavia, and in the United States, have called attention to the close resemblance between skeletal fluorosis and scurvy. This deserves much closer study, especially when we remember that a Vitamin C deficiency is fairly common in the Western World. It would not be too surprising to find that people who are Vitamin C deficient are more susceptible to fluoride-induced ill-effects. Unfortunately, there seems to be little awareness of this in the Western World.

Leningrad's Dr G S Konikova has devoted two scientific articles to his results showing that fluoride intake increases the level of blood cholesterol, the so-called "killer" in heart disease. Another Leningrad worker, Dr Y A Federov may well have suggested the ideal solution to the entire fluoride controversy: he has demonstrated that the compound *calcium glycerophosphate is twice as effective as fluoride for the reduction of dental caries*. The attractiveness of this finding is that calcium glycerophosphate is non-toxic, so would present none of the problems encountered with fluoride. Although the original Federov report came out in 1961, there is still no sign that follow-up research is being done anywhere in the Western World. Should we not make every effort to study this non-toxic alternative to fluoride?

In a 1966 article devoted to this general subject, Clair Nader asked: "If scientific evidence (eventually) should go against fluoridation, would scientists have the courage to retract, especially when the problem has generated so much emotion? And what would their retraction do to the public image of scientific prescience?"

In his last word, Clair Nader has hit upon the "nub" of fluoridation's biggest dilemma. Most of the basic concepts attending the "launching" of the fluoridation program, some 20 years ago, were based on prescience. At that time, there were almost no data regarding the comportment of fluoride in cases of specific illnesses. Lacking these data, the opinion prevailing at the time seems to have been motivated by a blissful wish that "all would go well". However, the time has now arrived for reckoning, for a "facing of facts", unpalatable though they may be. Although the world has not yet learned the lesson in the field of diplomacy, a loss of face should never be as important as a saving of lives. And yet, one cannot help but wonder just *when* this attitude will come about. In 1963, a large volume appeared bearing the title "*Fluorine Chemistry, Volume 4*". Although it is entirely devoted to the subject of fluorine (and fluoridation) it makes no mention of the cases cited in this summary, nor does it discuss any of the conditions presented here. This is so, even though the authors of the book have *themselves* presented some of this evidence in the past. Are these health problems to be conveniently buried and forgotten? Is the same lack of consideration to be accorded those human beings who react adversely to fluoride? If this is symptomatic of modern research, someone who has spent more than two decades in a research laboratory might well wonder just *where* Science is going.

The abstracts on pages 215-217 are grouped together because they comment, from various perspectives, on a fundamental change reported in the literature on water fluoridation. Once reported to be "perfectly safe", fluoridation is now held to *provide a benefit which "outweighs" admitted risks. - Editor*

AN ANALYSIS AND MONITORING REPORT WATER FLUORIDATION IN NEW ZEALAND *

Public Health Commission, Rangapu Hauora Tumatanui
Wellington, New Zealand, July 1994

Extracts from Executive Summary

It is estimated that the lifetime benefit for the average New Zealander drinking fluoridated water is the prevention of a total of 2.4 to 12.0 decayed missing or filled teeth. At a population level it is likely that water fluoridation prevents between 58,000 and 267,000 decayed, missing or filled teeth in New Zealand per year (with 50 percent of the population drinking fluoridated water).

It is possible that there is a small increased risk of hip fracture associated with water fluoridation, though the evidence for this is very inconclusive. More research is needed to clarify this issue. A large amount of research has failed to provide evidence that exposure to fluoride causes cancer. However, the possibility of a small increased risk of osteosarcoma (a rare type of bone cancer) in young men cannot be ruled out at this stage. Here again, more research is needed.

Aspects of the controversy over water fluoridation have probably led to some loss of public trust in public health authorities and dental professionals. This could have possible adverse effects on public trust and participation in other health related programmes that require complex risk/benefit assessments.

Comparing the health benefits and costs are difficult as various levels of risk that are unknown are necessary for the comparison. These risks are both the unquantified risks of dental abscesses and more serious infections (*eg*, infective endocarditis) and the uncertainty regarding possible low level risks of hip fractures or the more remote possibility of a risk of bone cancer. Given the current state of knowledge, most health professionals have put significant weight on the overall benefits of water fluoridation in terms of improving oral health (the principle of beneficence) and in achieving equity of health status outcomes. These factors have, in their view, outweighed the relatively low risk of harm and the minor reduction in individual autonomy for some citizens. However, members of the public may have less regard for the benefits of fluoridation and more concerns about individual rights and unknown risks.

A high degree of informed public input into deciding about water fluoridation is critical. This is not only for democratic reasons, but because value judgements are so important in considering what weight to put on possible, but unknown risks. Possibly the best mechanisms to achieve informed citizen participation are either citizens' panels or mixed citizen/expert panels as opposed to referenda. Generally, however, there remains a high requirement for further information on the benefits and costs of fluoridation to ensure a properly informed debate and to minimise the levels of uncertainty.

*For review and earlier editorial comment see *Fluoride 28* (1) 33-36 and (2) 57 1995.

SCIENCE OR FICTION?

John Colquhoun
Auckland, New Zealand

Abstract of letter published in New Zealand media in 1995

Our Public Health Commission (PHC) in its latest report claims that fluoridated water is estimated to prevent "a total of 2.4 to 12.0 decayed, missing or filled teeth" in a lifetime. On closer reading one learns that their calculation was made, not from New Zealand information, but from "data and assumptions" presented by one Ernest Newbrun of San Francisco, a leading US proponent of fluoridation. The calculation was made, not from New Zealand data, but solely from estimates of "effectiveness of fluoridation assumed" by Professor Newbrun from his interpretation of fluoridationists' claims from around the world.*

Even more shocks are in store for a careful reader. The PHC's estimate is followed by a graph, compiled from *New Zealand* school dental clinic records, showing the dramatic decline in tooth decay which has occurred among New Zealand 12- and 13-year-old children since 1977. The report omits to mention that the same decline has occurred in both fluoridated and non-fluoridated areas, and was actually slightly greater in the latter. It also omits to mention that the same school dental clinic records reveal that there is virtually no difference in tooth decay rates between the two kinds of area. In any case, the children in fluoridated areas had, by 1977, drunk fluoridated water most of their lives (fluoridation started mostly in the 1960s) so the decline since then could not be caused by fluoridation.

* The paper containing Newbrun's assumptions (*Journal of Public Health Dentistry 49* No.5 Special Issue 279-289 1989) omitted the large-scale New Zealand surveys showing no dental benefit from fluoridation (*Community Dentistry and Oral Epidemiology 13* 37-41 1985, *Community Health Studies 11* 85-90 1987).

FLUORIDE - HOW MUCH OF A GOOD THING? INTRODUCTION TO THE SYMPOSIUM

B A Burt
Ann Arbor, Michigan, USA.

Abstract from *Journal of Public Health Dentistry 55* (1)37-38 1995

January 25, 1995, is the 50th anniversary of the first controlled addition of fluoride to a public water supply. Those 50 years have seen extraordinary advances in oral health and consequent quality of life, for which fluoride use is generally considered the primary reason. More extensive exposure to fluoride in the modern era, however, has led to both a continuing decline in caries experience and an increased prevalence of dental fluorosis in children. At the other end of life, fluoride's role in bone strength among older people is not well defined. This symposium examines several aspects of fluoride use in the United States today, and has the purpose of helping to define the balance between maximizing the benefits of fluoride while minimizing its undesirable side effects.

Key words: Bone; Caries; Fluoride; Fluorosis; Symposium.

Reprints: B A Burt, Program in Dental Public Health, School of Public Health, University of Michigan, Ann Arbor, MI 48109-2029, USA.

COMMENTARY ON AND RECOMMENDATIONS FOR THE PROPER USES OF FLUORIDE

H S Horowitz
Bethesda, Maryland, USA

Abstract from *Journal of Public Health Dentistry 55* (1) 57-62 1995

Fluorosis has been associated with the fluoride concentration of drinking water, use of dietary fluoride supplements, early use of dentifrices, and prolonged use of infant formula. The literature, however, does not show associations between fluorosis and use of fluoride mouthrinses, professionally applied fluorides, bottled waters, carbonated beverages, and juices. It is unwise to issue laundry lists of items that may be implicated as problem-causing when, in fact, they may not be. Although usually classified without fluorosis, children in Dean's "questionable" category would be classified with the condition if the TFI or TSIF were used. Accordingly, Dean, in 1942, really reported only 52.8 percent of children without fluorosis in Kewanee, a community with 0.9 ppm fluoride in drinking water. Because the morbidity and sequelae of dental caries have declined, undue emphasis has been placed recently on the risks of using fluoride rather than on its profound beneficial effects. Although of paramount importance, conclusions cannot be drawn on whether fluoride protects against, contributes to, or has no effect on bone fractures or is valuable in treating osteoporosis. Careful thought is required before making recommendations that may reduce health benefits because of unfounded concerns about perceived risks. There should be greater regulation of extraneous fluoride sources, rather than reliance on educational efforts or recommendations to eliminate use of highly effective preventive regimens.

Key words: Benefits; Fluorosis; Pediatric toothpastes; Regulations; Supplements.
Reprints: H S Horowitz, 6307 Herkos Court, Bethesda, MD 20817 USA.

SOURCES OF FLUORIDE INTAKE IN CHILDREN

S M Levy, M C Kiritsy and J J Warren
Iowa City, Iowa, USA

Abstract from *Journal of Public Health Dentistry 55* (1) 39-52 1995

Wide variations in fluoride intake among children make estimating fluoride intake difficult. This paper discusses the various sources of fluoride intake among children, beginning with a review of the fluoride concentrations of water and other beverages, foods, and therapeutic fluoride products. A review of previous studies' estimates of fluoride intake from diet, dentifrice, fluoride supplements, fluoride mouthrinses, and gels, as well as total fluoride intake also is presented. Then, estimates of fluoride intake among young children of different age groups are summarized, and examples demonstrating the high level of variability of fluoride intake, both from individual sources and in total, are presented. Lastly, this paper discusses the implications of our current level of knowledge of children's fluoride intake, and presents recommendations for the use of fluoride for children in light of this current knowledge. The major recommendations are that: (1) the fluoride content of foods and beverages, particularly infant formulas and water used in their reconstitution, should continue to be monitored closely in an effort to limit

excessive fluoride intake: (2) ingestion of fluoride from dentifrice by young children should be controlled, and the use of only small quantities of dentifrice by young children should be emphasized; and (3) dietary fluoride supplements should be considered a targeted preventive regimen only for those children at higher risk for dental caries and with low levels of ingested fluoride from other sources.

Key words: Dentifrice; Diet; Exposure; Fluoride supplements; Ingestion; Intake.
Reprints: S M Levy, Department of Preventive and Community Dentistry, College of Dentistry, University of Iowa, Iowa City IA 52242, USA.

THE CARIOSTATIC MECHANISMS OF ACTION OF FLUORIDES. A REVIEW

C Fischer, A Lussi and P Hotz
Berlin, Germany

Abstract from *Schweizer Monatsschrift fur Zahnmedizin 105* (3) 311-317 1995

In the past the inhibition of caries by fluorides was ascribed to the reduced solubility of enamel due to the incorporation of F^- into the enamel mineral. During the last years the understanding of the cariostatic mechanism has changed fundamentally. Based on these new findings the loosely bound fluorides, which are present in the surroundings of the teeth after application of topicals, are regarded as decisive for the caries preventing effect by causing an inhibition of demineralization, enhancing the remineralization process and supporting the precipitation of CaF_2. The formation of CaF_2 is induced after application of topicals, and the material stays relatively stable in the mouth, due to adsorbed HPO_4^{2-} ions at the surface of CaF_2. During the cariogenic challenge, CaF_2 releases F^- ions due to the reduced concentration of HPO_4^{2-} ions at acidic pH values. The CaF_2 therefore functions as a pH-controlled F^--reservoir and is the most important supplier of free F^- ions during the cariogenic challenge.

Key words: Calcium fluoride; Cariostatic mechanisms; Dental caries; Remineralization.
Reprints: C Fischer, Klinik fur Zahnerhaltung, Universitat Bern, Berlin, Germany.

CHARACTERIZATION OF FLUOROSED HUMAN ENAMEL BY COLOR REFLECTANCE, ULTRASTRUCTURE, AND ELEMENTAL COMPOSITION

N J Giambro, K Prostak and P K Denbesten
Rochester, New York, USA

Abstract from *Caries Research 29* (4) 251-257 1995

Mature fluorosed human enamel has been described as a subsurface enamel hypomineralization, with porosity increasing relative to the degree of fluorosis. The purpose of the current study was to quantitatively measure the color of the fluorosed enamel by light reflectance, and to further characterize the enamel by scanning electron microscopy. Teeth with varying degrees of fluorosis were obtained and divided in groups of mild, moderate and severe fluorosis using Dean's

index for fluorosis. The color of the labial enamel surface was measured using a Minolta Chroma Meter CR241 (Minolta, Ramsey, N.J., USA). The teeth were further characterized for elemental composition using an energy-dispersive spectrometer, and imaged in both secondary and backscattered electron modes. The results of this study showed that the moderately and severely fluorosed enamel contained an uneven distribution of areas which were more electron-absorbent with a relatively increased carbon content. The changes in the physical characteristics of the teeth could be quantitated by measurements of light reflectance. The color of the teeth was significantly different between groups, with all groups significantly different than normal.

Key words: Colorimeter; Dental fluorosis; Enamel morphology; Scanning electron microscopy.

Reprints: P K Denbesten, Eastman Dental Cente, 625 Elmwood Ave, Rochester NY 14620, USA.

STUDIES ON THE INFLUENCE OF FLUORIDE ON THE EQUILIBRATING CALCIUM PHOSPHATE PHASE AT A HIGH ENAMEL/ACID RATIO

E I F Pearce, M J Larsen and T W Cutress
Wellington, New Zealand

Abstract from *Caries Research 29* (4) 258-265 1995

Data obtained in a previous study suggested that brushite is the solubility-determining phase when enamel is first exposed to acid solution in a series of repeated equilibrations. Fluoride in solution might be expected to inhibit brushite formation, and experimental studies at low solid/solution ratio support this. We have now re-examined the effect at a very high ratio, in an attempt to mimic what happens in an enamel caries lesion. Powdered enamel was repeatedly exposed to HCl solution, 10-70 mmol/L, containing 2 ppm F, for 24 h, initially in a ratio of 1 g/3 mi. Ion activities were determined after 20 min and 24 h and potential plot diagrams constructed. In early repetitions the -log (Ca^{2+}) $(OH^-)^2$ vs. -log $(H^+)^3(PO_4^{3-})$ points tended to follow the brushite line, rather than the hydroxy-apatite (HAp) line which one would expect if enamel behaved as pure HAp. Solution F was below measurable limits after 20 min and F then had little influence on the brushite equilibrating phase. In later (>13) repetitions, points fell closer to the HAp line, with or without F added to the acid solution. However, added F, which was not then completely removed from solution, caused the slope of the regression line through the points to approach the Ca/P ratio of HAp, and therefore may have had a small effect in reducing the brushite phase. It is concluded that high solid/solution ratio, a previously neglected factor in enamel dissolution studies, has a profound effect in increasing the manifestation of a brushite surface phase and reducing the inhibitory effect off on this phase.

Key words: Brushite; Dental enamel; Fluoride.

Reprints: E I F Pearce, Health Research Council of New Zealand, Dental Research Unit, PO Box 27007, Wellington, New Zealand.

ENDEMIC FLUOROSIS IN SAN LUIS POTOSI, MEXICO I. IDENTIFICATION OF RISK FACTORS ASSOCIATED WITH HUMAN EXPOSURE TO FLUORIDE

M Grimaldo, V H Borja-Aburtom, A L Ramirez, M Ponce, M Rosas and F Diaz-Barriga.
San Luis Potosi, Mexico

Abstract from *Environmental Research 68* (1) 25-30 1995

In order to identify risk factors associated with human exposure to fluoride in San Luis Potosi (SLP), Mexico, a biochemical and epidemiological study was carried out in 1992. Results from the analysis of fluoride sources showed that 61% of tap water samples had fluoride levels above the optimal level of 0.7-1.2 ppm. The levels were higher after boiling. In bottled water, fluoride levels ranged from 0.33 to 6.97 ppm. These sources are important since in SLP 82% of the children drink tap water, 31% also drink bottled water, 92% prepare their food with tap water, 44% boiled all the drinking water, and 91% used infant formula reconstituted with boiled water. The prevalence and severity of dental fluorosis in children (11-13 years old) increased as the concentration of water fluoride increased. At levels of fluoride in water lower than 0.7 ppm a prevalence of 69% was found for total dental fluorosis, whereas at levels of fluoride in water higher than 2.0 ppm a prevalence of 98% was found. In the same children, fluoride levels in urine were quantified. The levels increased as the concentration of water fluoride increased. Regressional analysis showed an increment of 0.54 ppm ($P < 0.0001$) of fluoride in urine for each ppm of fluoride in water. Fluoride urinary levels were higher in samples collected during the afternoon (1800) when compared with sample collected during the morning (1100). Taking together all these results, three risk factors for human exposure to fluoride in SLP can be identified: ambient temperature, boiled water, and food preparation with boiled water. These factors explain the prevalence of dental fluorosis in SLP.

Key words: Dental fluorosis; Mexico; Risk factors.

Reprints: M Grimaldo, Facultad de Medicina, Universidad Autonoma de San Luis Potosi San Luis Potosi, Mexico.

Other articles related to dental effects of fluoride (including non-blind sample studies from selected communities, which continue to appear in the dental literature) are listed:

EFFECTS OF LIFELONG CONSUMPTION OF FLUORIDATED WATER OR USE OF FLUORIDE SUPPLEMENTS ON DENTAL CARIES PREVALENCE. D C Clark *et al. Community Dentistry and Oral Epidemiology 23* (1) 20-24 1995. Reprints: Department of Clinical Dental Services, University of British Columbia, 2199 Wesbrook Hall, Vancouver BC, Canada V6T IZ3.

THE ASSOCIATION BETWEEN AREA DEPRIVATION AND DENTAL CARIES IN GROUPS WITH AND WITHOUT FLUORIDE IN THEIR DRINKING WATER. R P Ellwood and D M O'Mullane *Community Dental Health 12* (1) 18-22 1995. Wilton, Cork, Ireland. Reprints: Dental Health Unit, Unit 3a, Skelton House, Manchester Science Park, Lloyd Street North, Manchester M15 6SH England.

THE PREVALENCE OF DENTAL CARIES IN SLOVENIA IN 1987 AND 1993. V L Vrbic. *Community Dental Health 12* (1) 39-41 1995. Reprints: Medical Faculty, Department of Stomatology, University of Ljubljana, Hrvatski trg 6, 61105, Ljubljana, Slovenia.

EFFECTS OF FLUORIDE ON RAT VERTEBRAL BODY BIOMECHANICAL COMPETENCE AND BONE MASS

C H Sogaard, L Mosekilde, W Schwartz,
G Leidig, H W Minne and R Ziegler
Aarhus, Denmark

Abstract from *Bone 16* (1) 163-169 1995

For more than 30 years, sodium fluoride has been a commonly used therapeutic agent for established osteoporosis because of its repeatedly documented anabolic effect on trabecular bone mass. Recent clinical and experimental studies have, however, indicated a possible detrimental effect of fluoride on bone strength. Thus, the efficacy of fluoride therapy remains a controversial issue. The aim of this study was to investigate the effect of fluoride on both vertebral bone mass and quality in rats. Twenty-nine 3-month-old female rats were randomized into three groups. One group served as a control group, and the other two groups received fluoridated water at different doses (100 ppm and 150 ppm). The rats were followed for 90 days. Three lumbar vertebrae were obtained from each rat, and changes in bone fluoride content, bone mass and biomechanical competence were assessed. The results revealed a significant increase in bone fluoride content, ash density and trabecular bone volume after fluoride treatment. Directly obtained load values and load corrected for cross-sectional area were constant. Load corrected for ash content, which is a measure of bone quality, decreased significantly after fluoride therapy. It is concluded that the increase in bone mass during fluoride treatment does not translate into an improved bone strength and that the bone quality declines. This investigation thereby supports the hypothesis of a possible negative effect of fluoride on bone quality.

Key words: Bone mass; Bone strength; Fluoride therapy; Rat.
Reprints: C H Søgaard, Herluf Trolles Gade 7B, st tv, DK-8200, Aarhus N, Denmark.

RELATIONSHIP BETWEEN BONE FLUORIDE CONTENT, PATHOLOGICAL CHANGE IN BONE OF ABORTED FETUSES, AND MATERNAL FLUORIDE LEVEL

J Shi, G Dai and Z Zhang.
Shenyang, China

Abstract from *Chung-Hua Yu Fang i Hsueh Tsa Chih* 29 (2) 103-105 1995

Relationship between bone fluoride content, pathological change in bone of aborted fetuses, and maternal fluoride level was studied in 46 pregnant women and their induced-aborted fetuses. Results showed fluoride content in fetal femur averaged 368.2 micrograms/g, and 41.4% of the bone with pathological change. Fluoride levels in maternal urine and amniotic fluid, and fluoride content in fetal femur and pathological change in fetal femur, appeared to have a positive correlation. Femur fluoride content, and pathological change of bone, in fetuses born to mothers with mottled teeth, were significantly greater than to those without them. Pathological change in fetal femurs had a dose-response relationship with their bone fluoride content. When the latter reached greater than 500 micrograms/g, pathological changes occurred in 90% of the bones.

Key words: Bone; Fetus; Fluoride.
Reprints: J Shi, Department of Epidemiology, China Medical University, Shenyang, China.

A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR FLUORIDE UPTAKE BY BONE

H V Rao, R P Beliles, G M Whitford and C H Turner
Hartford, Connecticut, USA

Abstract from *Regulatory Toxicology and Pharmacology 22* (1) 30-42 1995

A sex-specific, physiologically based pharmacokinetic (pbpk) model has been developed to describe the absorption, distribution, and elimination of fluorides in rats and humans. Growth curves generated by plotting mean body weights (kg) against age (weeks or years) are included in the simulation model to allow the integration of chronic fluoride exposure from birth to old age. The model incorporates age and body weight dependence of the physiological processes that control the uptake of fluoride by bone and the elimination of fluoride by the kidneys. Six compartments make up the model. These are lung, liver, kidney, bone, and slowly and rapidly perfused compartments. The model also includes two bone subcompartments: a small, flow-limited, rapidly exchangeable surface bone compartment and a bulk virtually nonexchangeable inner bone compartment. The inner bone compartment contains nearly all of the whole body content of fluoride, which, in the longer time frame, may be mobilized through the process of bone modeling and remodeling. The model has been validated by comparing the model predictions with experimental data gathered in rats and humans after drinking water and dietary ingestion of fluoride. This physiological model description of absorption, distribution, and elimination of fluoride from the body permits the analysis of the combined effect of ingesting and inhaling fluorides on the target organ, bone. Estimates of fluoride concentrations in bone are calculated and related to chronic fluoride toxicity. The model is thus useful for predicting some of the long-term metabolic features and tissue concentrations of fluoride that may be of value in understanding positive or negative effects of fluoride on human health. In addition, the pbpk model provides a basis for across-species extrapolation of the effective fluoride dose at the target tissue, bone, in the assessment of risk from different exposure conditions.

Key words: Bone; Fluoride uptake; Pharmacokinetic model.

Reprints: H V Rao, Department of Public Health and Addiction Services, 150 Washington St, Hartford CT 06106, USA.

FLUORIDE AND BONE HEALTH

K Phipps
Newport, Oregon, USA

Abstract from *Journal of Public Health Dentistry 55* (1) 53-56 1995

This paper reviews some of the studies related to the effect of fluoride on the skeletal system of humans and outlines the knowns and unknowns of fluoride and bone health. Current research indicates that, in large enough doses, fluoride stimulates bone formation by osteoblastic stimulation, increases bone formation earlier and to a larger extent in trabecular bone compared to cortical bone, and increases spinal bone density. There is controversy, however, concerning the efficacy of fluoride as a therapeutic agent in the treatment of osteoporosis. Some clinical studies have found a reduction in vertebral fracture rates while others have not. To date,

only ecologic studies have been conducted on the association between water fluoridation and hip fractures. The inability of ecologic studies to control for confounding variables makes their interpretation difficult. Based on the literature presented, it is concluded that there are more unknowns than knowns in terms of fluoride's effect on bone, osteoporosis, and fractures. One of the major unknowns in the relationship between fluoride and bone health is dose and duration. Two studies are under way that attempt to describe the dose-response relationship between waterborne fluoride and osteoporosis. These studies will be completed in the near future and their results, while providing new insight into fluoride's effects on bone, will by no means answer all the questions raised on this issue.

Key words: Bone; Fluoride; Osteoporosis; Research; Review.

Reprints: K Phipps, Oregon Health Sciences University, 131 NW 20th , Suite B, Newport OR 97365, USA.

RELATIONSHIP BETWEEN FLUORIDE CONTENT IN BONES AND THE AGE OF EUROPEAN ELK (*ALCES ALCES L*)

Z Machoy, E Dabkowska, D Samujlo, T Ogonski, J Raczynski and Z Gebczynska
Szczecin, Poland

Abstract from *Comparative Biochemistry and Physiology C - Pharmacology, Toxicology & Endocrinology 111* (1) 117-120 1995

The relation between fluoride content in bones (jaws) and the age of elks living in Poland in areas of relatively low pollution by industrial emissions was studied. Multiple regression analysis was performed by making use of 11 mathematical models. The relationship between fluoride content in bones and the animals age is best described by square root and linear models.

Key words: Air fluoride; Animal age; Bone; Environment; European elk; Fluoride accumulation; Lower jaw; Mineral composition.

Reprints: Z Machoy, Pomeranian Medical Academy, Department of Biochemistry, Al Powstanców Wielkopolskich 72, PL-70111 Szczecin, Poland.

DIAGNOSIS AND TREATMENT OF OSTEOPOROSIS

C Alexandre
St Etienne, France

Abstract from *Current Opinion in Rheumatology* 7 (3) 240-242 1995

A new definition of osteoporosis has been proposed by an expert panel of the World Health Organization, in which cut-off diagnostic and therapeutic values are derived from bone mass measurements, leading to the practical idea of densitometric osteoporosis with or without fractures. At the individual level, risk factors of bone fragility are not accurate enough to allow definition of an "at risk" population. Thus, bone densitometry remains the major parameter to be analyzed. In terms of therapy, all protocols refer to the more academic definition of osteoporosis, so that no true innovation appeared in the literature over the past year. The roles of calcium, exercise, hormone replacement therapy, calcitonin, fluoride, and bisphosphonates are more accurately defined in established osteoporosis. However, new studies are needed in the investigation of densitometric osteoporosis. More data are needed, particularly because the biomechanical qualities of the bone

remain questionable after treatment with fluoride. A 45% to 58% reduction was noted at the trabecular level for bone strength and bone quality, respectively, in patients treated for 5 years with 40 to 60 mg/d sodium fluoride.

Key words: Definition; Fluoride; Osteoporosis.

Reprints: C Alexandre, Service de Rhumatologie, Hôpital Bellevue, Boulevard Pasteur, 42055 Saint Etienne Cedex, France.

HISTOMORPHOMETRIC ANALYSIS OF ILIAC CREST BONE BIOPSIES IN PLACEBO-TREATED VERSUS FLUORIDE-TREATED SUBJECTS

M W Lundy, M Stauffer, J E Wergedal, D J Baylink, J D Featherstone, S F Hodgson and B L Riggs.
Loma Linda, California, USA

Abstract from *Osteoporosis International* 5 (2) 115-129 1995 .

In a 4-year controlled, prospective trial, histomorphometric analysis was used to compare the tissue-level skeletal effects of fluoride therapy in 43 postmenopausal women (75 mg NaF/day) with those of 35 matching placebo subjects; all subjects received 1500 mg/day elemental calcium supplement. In addition to an initial, baseline biopsy, a second biopsy was obtained after 6, 18, 30 or 48 months. Measurements were made on a third biopsy obtained from 8 subjects following at least 72 months of fluoride therapy. The change in cancellous bone volume or trabecular thickness in fluoride-treated subjects was not different from a change in placebo-treated subjects. However, paired analysis in the fluoride-treated subjects indicated that bone volume was increased between the first and second biopsies ($p < 0.005$). Both osteoid length and width were significantly increased in fluoride compared with placebo subjects; however, only the osteoid surface increased linearly ($r = 0.63$, $p < 0.001$). The mineral apposition rate and relative tetracycline-covered bone surface were not different between fluoride and placebo treatment, although they were decreased in both groups in the second biopsy. The tetracycline-covered bone surface returned to normal in the third biopsy. Definitive evidence for osteomalacia is a prolonged mineralization lag time, which following fluoride treatment was found to be increased 9-fold in the second biopsy and 4-fold in the third biopsy. Further evidence for osteomalacia was increased osteoid thickness by 6 months, evidence of focal areas of interstitial mineralization defects, and broad tetracycline labels of low fluorescence intensity. In the third biopsies, osteoclastic resorption was observed beneath osteoid seams. Fluoride therapy increased the cortical width compared with placebo treatment ($p < 0.02$), and increased the osteoid surface in Haversian canals, but did not change the osteoid width, resorption surface or cortical porosity. After an initial rise, serum fluoride levels remained constant, and the urine values fell slightly. The bone fluoride concentration rose throughout the treatment period, and was correlated with the change in osteoid-covered bone surface ($r = 0.56$, $p < 0.001$). Although we found definitive evidence for osteomalacia, the cause of the osteomalacia was not determined in this study. On the other hand, the presence of bone resorption beneath unmineralized osteoid and of osteocyte halos is suggestive of hyperparathyroidism. Thus, it is possible that the strong stimulus for bone formation brought about by fluoride therapy resulted in relative calcium deficiency.

Key words: Bone; Bone biopsy; Bone strength; Fluoride therapy.

Reprints: M W Lundy, Pettis Veterans Hospital, Loma Linda CA, USA.

RELATION OF EXPOSURE TO AIRWAY IRRITANTS IN INFANCY TO PREVALENCE OF BRONCHIAL HYPER-RESPONSIVENESS IN SCHOOLCHILDREN

V Soyseth, J Kongerud, D Haarr, O Strand, R Bolle and J Boe
Årdal, Norway

Abstract from *Lancet 345* (8944) 217-220 1995

To find out whether exposure to sulphur dioxide during infancy is related to the prevalence of bronchial hyper-responsiveness (BHR), we studied schoolchildren (aged 7-13 years) from two areas of Norway - a valley containing a sulphur-dioxide-emitting aluminium smelter and a similar but non-industrialised valley. Bronchial responsiveness was assessed in 529 of the 620 participants. The median exposures to sulphur dioxide and fluoride were 37.1 micrograms/m^3 and 4.4 micrograms/m^3 at ages 0-12 months and 37.9 micrograms/m^3 and 4.4 micrograms/m^3 at 13-36 months. The risk of BHR increased with exposure to sulphur dioxide and fluoride at these ages; the odds ratio for a 10 micrograms/m^3 increase in sulphur dioxide exposure at 0-12 months was 1.62 (95% CI 1.11-2.35) and that for a 1 microgram/m^3 increase in fluoride exposure was 1.35 (1.07-1.70) at 0-12 months and 1.38 (1.05-1.82) at 13-36 months. Exposure to these low concentrations of airway irritants during early childhood is associated with an increased prevalence of BHR in schoolchildren.

Key words: Bronchial hyper-responsiveness; Children; Fluoride; Sulphur dioxide.
Reprints: V Søyseth, Hydro Aluminium Årdal, N-5870, Øvre, Årdal, Norway.

ASTHMA AND RESPIRATORY PROBLEMS - A REVIEW.

T V O'Donnell
Wellington, New Zealand

Abstract from *Science of the Total Environment 163* (1-3) 137-145 1995

Occupational asthma is the principal respiratory health problem within the primary aluminium industry. Current evidence indicates that it is irritant induced and due to occupational exposure to the inhalation of gaseous or particulate fluoride compounds. Following transfer from the occupational exposure of those who develop asthma, there is commonly symptomatic improvement. A programme of compulsory respiratory protection, progressive engineering improvements and of regular screening of potroom workers aimed at early detection, and the transfer of asthmatic workers from that environment has resulted not only in improvement of asthmatic symptoms among them, but also in the majority of an improvement in bronchial responsiveness as assessed by methacholine inhalation. The majority of studies indicate a slightly increased prevalence of symptoms of chronic bronchitis and of chronic obstructive pulmonary disease among workers in carbon bake areas, although tobacco smoking has a greater and additive effect. Only a trivial number of clinical cases of pulmonary fibrosis ascribed to aluminium compounds has been reported. Particle size limits smelter grade primary alumina reaching the alveoli of the lung.

Key words: Aluminum, Asthma; Occupational fluorosis; Potroom.
Reprints: Professor T V O'Donnell, University of Otago, Wellington School of Medicine, Wellington, New Zealand.

EFFECT OF DIFFERENT EXPOSURE COMPOUNDS ON URINARY KINETICS OF ALUMINIUM AND FLUORIDE IN INDUSTRIALLY EXPOSED WORKERS

F Pierre, F Baruthio, F Diebold and P Biette
Vandoeuvre Nancy, France

Abstract from *Occupational and Environmental Medicine 52* (6) 396-403 1995

OBJECTIVE: To conduct a field study to obtain information on the urinary concentrations of aluminium (Al) and fluoride (F^-) depending on the different compounds exposed to in the aluminium industry.

METHODS: 16 workers from one plant that produced aluminium fluoride (AlF_3), and from two plants that produced aluminium electrolytically by two different processes participated in the study for one working week. Pollutants were monitored by eight hour personal sampling every day, and urine samples were collected during the week. Al and F^- were analysed in both atmospheric and urine samples by atomic absorption spectrometry and an ion selective electrode.

RESULTS: The principal results show different characteristics of kinetic curves of Al and F^- excretion in workers with different exposures. Some characteristics of excretory peaks were linked to specific exposures-for instance, after exposure to AlF_3 there was one delayed Al peak associated with one delayed F^- peak about eight hours after the end of the daily shift, and after mixed exposure to HF and AlF_3, two F^- peaks were noted, one fast peak at the end of the shift and another delayed peak at 10 hours synchronised with an Al peak. In one of the electrolysis plants, the exposure to Al and F^- compounds led to the simultaneous excretion of Al and F^- peaks, either as a single peak or two individual ones depending on the type of technology used on site (open or enclosed potlines). The average estimated half life of Al was 7.5 hours, and of F^- about nine hours. Quantitative relations between excretion and exposure showed an association between the F^- atmospheric Limit value of 2.5 mg/m^3 with a urinary F^- concentration of 6.4 mg/g creatinine at the end of the shift, a peak of 7.4 mg/g creatinine, and 7.4 mg excreted a day. For Al, the exposure to 1.36 mg/m^3 during the shift corresponded to a urinary concentration at the end of the shift of 200 mu g/g creatinine. Daily excretion of 200 mu g corresponded to an exposure to 0.28 mg/m^3.

Conclusion - Particular differences in the behaviour of Al and F^- in urine depended upon the original molecular form in the pollutant. These results reinforce the principle that, in biological monitoring, the sampling strategy and the choice of limit value should be dependent on kinetic data that take the exposure compound of the element in question into account.

Key words: Aluminium; Biological monitoring; Fluoride.
Reprints: F Pierre, Ave Bourgogne BP 27, F-54501 Vandoeuvre Nancy, France.

SHIBBOLETHS AND JIGSAW PUZZLES: THE FLUORIDE NEPHROTOXICITY ENIGMA

B R Brown
Tucson, Arizona, USA

Abstracted from Editorial in *Anesthesiology 82* (3) 607-608 1995

In this issue of the Journal, Kharasch *et al.* postulate that intrarenal production of fluoride ion may be a more important factor for nephrotoxicity than hepatic metabolism, which causes increased plasma fluoride ion levels. Varying cytochrome P-450 isoforms with differing rates of intrarenal biotransformation would help solve the genetic issue. Substantiation of the hypothesis would explain why patients can have an increased concentration of plasma fluoride after receiving drugs such as isoflurane and sevoflurane without evidence of nephrotoxicity. The major thesis that nephrotoxicity is agent-specific and occurs primarily because of intrarenal fluoride ion production and is not primarily dependent on fluoride ion plasma concentration is impressive. It underscores the rule that medicine can never rest on its laurels. Each day brings us new knowledge from investigations, humble and profound, mundane and exotic, and we must learn to discard old indoctrinated mechanisms when they no longer stand the tests of time.

Key words: Anesthesia; Fluoride; Isoflurane; Mechaism; Nephrotoxicity; Sevoflurane.
Reprints: B R Brown, Department of Anesthesiology, University of Arizona, Tucson AZ 85724, USA.

HUMAN KIDNEY METHOXYFLURANE AND SEVOFLURANE METABOLISM. INTRARENAL FLUORIDE PRODUCTION AS A POSSIBLE MECHANISM OF METHOXYFLURANE NEPHROTOXICITY

E D Kharasch, D C Hankins and K E Thummel
Seattle, USA

Abstract from *Anesthesiology 82* (3) 689-699 1995

BACKGROUND: Methoxyflurane nephrotoxicity is mediated by cytochrome P450-catalyzed metabolism to toxic metabolites. It is historically accepted that one of the metabolites, fluoride, is the nephrotoxin, and that methoxyflurane nephrotoxicity is caused by plasma fluoride concentrations in excess of 50 microM. Sevoflurane also is metabolized to fluoride ion, and plasma concentrations may exceed 50 microM, yet sevoflurane nephrotoxicity has not been observed. It is possible that in situ renal metabolism of methoxyflurane, rather than hepatic metabolism, is a critical event leading to nephrotoxicity. We tested whether there was a metabolic basis for this hypothesis by examining the relative rates of methoxyflurane and sevoflurane defluorination by human kidney microsomes.

METHODS: Microsomes and cytosol were prepared from kidneys of organ donors. Methoxyflurane and sevoflurane metabolism were measured with a fluoride-selective electrode. Human cytochrome P450 isoforms contributing to renal anesthetic metabolism were identified by using isoform-selective inhibitors and by Western blot analysis of renal P450s in conjunction with metabolism by individual P450s expressed from a human hepatic complementary deoxyribonucleic acid library.

RESULTS: Sevoflurane and methoxyflurane did undergo defluorination by human kidney microsomes. Fluoride production was dependent on time, reduced nicotinamide adenine dinucleotide phosphate, protein concentration, and anesthetic concentration. In seven human kidneys studied, enzymatic sevoflurane defluorination was minima, whereas methoxyflurane defluorination rates were substantially greater and exhibited large interindividual variability. Kidney cytosol did not catalyze anesthetic defluorination. Chemical inhibitors of the P450 isoforms 2E1, 2A6, and 3A diminished methoxyflurane and sevoflurane defluorination. Complementary deoxyribonucleic acid-expressed P450s 2E1, 2A6, and 3A4 catalyzed methoxyflurane and sevoflurane metabolism, in diminishing order of activity. These three P450s catalyzed the defluorination of methoxyflurane three to ten times faster than they did that of sevoflurane. Expressed P450 2B6 also catalyzed methoxyflurane defluorination, but 2B6 appeared not to contribute to renal microsomal methoxyflurane defluorination because the P450 2B6-selective inhibitor had no effect.

CONCLUSIONS: Human kidney microsomes metabolize methoxyflurane, and to a much lesser extent sevoflurane, to fluoride ion. P450s 2E1 and/or 2A6 and P450 3A are implicated in the defluorination. If intrarenally generated fluoride or other metabolites are nephrotoxic, then renal metabolism may contribute to methoxyflurane nephrotoxicity. The relative paucity of renal sevoflurane defluorination may explain the absence of clinical sevoflurane nephrotoxicity to date, despite plasma fluoride concentrations that may exceed 50 microM.

Key words: Anesthesia; Fluoride; Methoxyfluorane; Nephrotoxicity; Sevofluorane.

Reprints: E D Kharasch, Department of Anesthesiology, RN-10, University of Washington, Seattle WA 98195, USA.

RENAL FUNCTION AND SERUM FLUORIDE CONCENTRATIONS IN PATIENTS WITH STABLE RENAL INSUFFICIENCY AFTER ANESTHESIA WITH SEVOFLURANE OR ENFLURANE

P F Conzen, M Nuscheler, A Melotte, M Verhaegen, T Leupolt, H Vanaken and K Peter
Munich, Germany

Abstract from *Anesthesia and Analgesia 81* (3) 569-575 1995

Sevoflurane is metabolized to hexa-fluoro-isopropanol and inorganic fluoride by the human liver. Its use as an anesthetic may lead to peak plasma fluoride concentrations exceeding those seen after enflurane. Although there is no nephrotoxicity after sevoflurane anesthesia in humans with normal kidneys, those with

chronically impaired renal function might be at increased risk because of increased fluoride load due to prolonged elimination half-life. In this study, measures of renal function after sevoflurane anesthesia were compared to those after enflurane in patients with chronically impaired renal function. Forty-one elective surgical patients with a stable preoperative serum creatinine concentration greater than or equal to 1.5 mg/dL were randomly allocated to receive sevoflurane (n = 21) or enflurane (n = 20) at a fresh gas inflow rate of 4 L/min for maintenance of anesthesia. Serum fluoride concentrations were measured by ion-selective electrode. Renal function (creatinine, urea, sodium,osmolality) was assessed in serum and urine preoperatively and for up to 7 days postoperatively. Peak serum inorganic fluoride concentrations were significantly higher after sevoflurane than after enflurane anesthesia (25.0 ± 2.2 vs 13.3 ± 1.1 μM; mean ± SEM). Laboratory measures of renal function remained stable throughout the postoperative period in both groups. No patient suffered a permanent deterioration of preexisting renal insufficiency and none required dialysis. Thus, neither sevoflurane nor enflurane deteriorated postoperative renal function in these patients with preexisting renal insufficiency. There is no evidence that fluoride released by metabolism of sevoflurane metabolism worsened renal function in these patients with stable, permanent serum creatinine concentrations more than 1.5 mg/dL. Our data also suggest that the peak fluoride concentrations measured in peripheral blood may not be a good predictor of nephrotoxic potential after sevoflurane anesthesia in these patients.

Key words: Anesthesia; Fluoride; Methoxyfluorane; Nephrotoxicity; Sevofluorane.
Reprints: P F Conzen, University of Munich, Klinikum Grosshadern, Institute of Anesthesiology, Marchioninistr 15, D-81377 Munich, Germany.

SERUM HAPTOGLOBIN AND C-REACTIVE PROTEIN IN HUMAN SKELETAL FLUOROSIS

A K Susheela and P Jethanandani
New Delhi, India

Abstract from *Clinical Biochemistry* 27 (6) 463-468 1994

Circulating levels of haptoglobin and C-reactive protein were studied in patients with skeletal fluorosis and compared with two types of controls. The first type of control included normal healthy individuals consuming water containing permissible levels of fluoride (up to 1 mg/L). The second type of control included individuals consuming water contaminated with fluoride (1.2-14.5 mg/L) but not exhibiting clinical manifestations of skeletal fluorosis. A significant increase in the levels of haptoglobin ($p < 0.01$) and C-reactive protein ($p < 0.01$) as well as a raised erythrocyte sedimentation rate were seen in patients with skeletal fluorosis as compared to both types of controls. The present study suggests the possibility of a subclinical inflammatory reaction occurring in patients with skeletal fluorosis.

Key words: Erythrocyte sedimentation rate; C-reactive protein; Haptoglobins; Skeletal fluorosis.
Reprints: A K Susheela, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India.

SAFETY OF CIPROFLOXACIN THERAPY IN CHILDREN: MAGNETIC RESONANCE IMAGES, BODY FLUID LEVELS OF FLUORIDE AND LINEAR GROWTH

K M Pradhan, N K Arora, A Jena, A K Susheela and M K Bhan
New Delhi, India

Abstract from *Acta Paediatrica 84* (5) 555-560 1995

We evaluated the safety of ciprofloxacin administered in a dose of 15-25 mg/kg for 9-16 days, in a case series of 58 children who were between 8 months and 13 years of age. No arthropathy was observed during therapy and follow-up. Blinded evaluation of 22 pairs of nuclear magnetic resonance scans obtained before and between day 10 and 15 of therapy did not reveal any cartilage damage. After the first dose of ciprofloxacin (10 mg/kg), serum fluoride levels increased at 12 h in 15 of 19 (79%) patients; 24-h urinary fluoride excretion was higher on day 7 compared with basal values in 16 of 18 (88.9%) patients. Height z scores of 53 patients at a mean of 22.5 months of follow-up were not significantly different from basal scores ($p = 0.12$). In conclusion, ciprofloxacin may be recommended for use in children for short duration when effective alternative antibacterials are unavailable. However, there is a need for further studies to evaluate the tissue accumulation of fluoride and its potential to cause toxic effects.

Key words: Children; Ciprofloxacin toxicity; Fluoride; Linear growth; NMR images.
Reprints: K M Pradhan, Department of Paediatrics; All India Institute of Medical Sciences, New Delhi 110029, India.

INHALATION ONCOGENICITY BIOASSAY IN RATS AND MICE WITH VINYL FLUORIDE

M S Bogdanffy, G T Makovec and S R Frame
Newark, Delaware, USA

Abstract from *Fundamental and Applied Toxicology 26* (2) 223-238 1995

The purpose of this study was to assess the oncogenic potential of vinyl fluoride in rats and mice when administered by inhalation. Male and female rats and mice were exposed to 0, 25, 250, or 2500 ppm vinyl fluoride 6 hr per day, 5 days per week, for 2 years (rats) or 18 months (mice). Slight body weight gain decrements were noted in groups of vinyl fluoride-exposed rats and mice. No significant clinical signs of toxicity were noted other than an increase in the incidence of palpable masses in the region of the mammary gland in female mice exposed to vinyl fluoride. Survival was decreased in male rats and mice of the 250 and 2500 ppm groups and female rats and mice of all vinyl fluoride-exposed groups compared to controls. Urinary fluoride excretion, an indicator of vinyl fluoride metabolism, increased with concentration and time although the dose relationship appeared to plateau at concentrations greater than or equal to 250 ppm. Gross observations made at necropsy of rats supported histological observations of hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, hepatic foci

of clear cell and basophilic alteration, hepatic sinusoidal dilatation, metastatic lung tumors, and Zymbal's gland tumors. Hepatic hemangiosarcoma was the sentinel lesion in rats. Gross observations made at necropsy of mice supported histological observations of bronchioloalveolar adenoma and hyperplasia, hepatic hemangiosarcoma and hepatocellular hyperplasia with angiectasis and peliosis, and mammary gland adenocarcinoma and hyperplasia. Bronchioloalveolar adenoma appeared to be the sentinel lesion in mice. The spectrum of vinyl fluoride-induced tumors is similar to that induced by other monohaloethylenes in rats and mice. Under the conditions of this study, vinyl fluoride was carcinogenic in male and female rats and mice at concentrations greater than or equal to 25 ppm.

Key words: Mice; Oncogenicity; Rats; Vinyl fluoride.
Reprints: M S Bogdanffy, Dupont Company Inc, Haskell Laboratory of Toxicology and Industrial Medicine, PO Box 50, Newark DE 19714, USA.

GRAMINE AND FREE AMINO ACIDS AS INDICATORS OF FLUORIDE-INDUCED STRESS IN BARLEY AND ITS CONSEQUENCES TO INSECT HERBIVORY

E L Hautala and J K Holopainen
Kuopio, Finland

Abstract from *Ecotoxicology and Environmental Safety 31* (3) 238-245 1995

Barley leaves were sprayed with aqueous NaF, which caused accumulation of fluoride in the foliage, but no visible symptoms were detectable. No significant correlation was observed between foliar fluoride concentration and content of the indole alkaloid gramine after exposure to fluoride levels of 20 to 60 mg F liter(-1). Fluoride exposure did not explicitly affect the performance of *Carausius morosus* or *Rhopalosiphum padi* on barley. After exposure to fluoride levels of 100 and 200 mg F liter(-1), as NaF, fluoride treatment had a significant effect on gramine concentration of the first leaf of barley, being highest at a fluoride treatment of 200 mg liter(-1), and there was a slight, but significant positive correlation between the log-transformed foliar fluoride concentration and log-transformed gramine concentration of the first leaf. Fluoride treatment increased levels of some individual free amino acids in barley foliage. Exposure of young barley to NaF in aqueous form caused accumulation of fluoride in barley foliage and resulted in increased levels of gramine in the first leaf and levels of some free amino acids in foliage. It is possible that the fluoride-induced concurrent increase in gramine concentration in barley leaves could override the eventual increase in nutritive value to herbivorous insects after fluoride exposure. More detailed biochemical studies of the induction of gramine production are needed to understand the fluoride effects in secondary metabolism of barley.

Key words: Amino acids; Barley; Gramine; Herbivorous insects; Sodium fluoride.
Reprints: E L Hautala, University of Kuopio, Department of Environmental Sciences, PO Box 1627, SF-70211 Kuopio, Finland.

PLANT UPTAKE OF FLUORIDE IN IRRIGATION WATER BY LADYFINGER (*ABELMORCHUS ESCULENTUS*)

V Singh, M K Gupta, P Rajwanshi, S Mishra, S Srivastava, R Srivastava, M M Srivastava, S Prakash and S Dass
Agra, Uttar Pradesh, India

Abstract from *Food and Chemical Toxicology 33* (5) 399-402 1995

Because of suggestions that food is a rich source of fluoride to humans and the absence of permissible and upper limits of fluoride for irrigation water, plant uptake studies were conducted using fluoride-rich irrigation water. Ladyfinger was grown in sand and soil cultures for 18 wk and the accumulation of fluoride in various plant parts was studied. The potential for ingestion of fluoride by humans through this route was also considered. The percentage uptake was greater in sand-cultured plants than in soil-cultured plants. The root accumulates most of the fluoride supplied through irrigation water and the fruit accumulates the least. Up to 120 mg fluoride/litre of irrigation water did not harm the plants. The ingestion of fluoride by humans from plants irrigated with water containing 10 mg fluoride/litre would be 0.20 mg per 100 g ladyfinger.

Key words: Fluoride uptake; Irrigation water; Ladyfinger; Plant uptake.
Reprints: S Dass, Dayalbagh Educational Institute, Agra 282005 UP, India.

FLUORIDE TOXICITY TO AQUATIC LIFE: A PROPOSAL OF SAFE CONCENTRATIONS FOR FIVE SPECIES OF PALEARCTIC FRESHWATER INVERTEBRATES

J A Camargo and T W Lapoint
Madrid, Spain

Abstract from *Archives of Environmental Contamination and Toxicology 29* (2)159-163 1995

Safe concentrations (SCs) and EC50s of fluoride ion (F^-) for five species of aquatic insect larvae, *Chimarra marginata*, *Hydropsyche bulbifera*, *H. exocellata*, *H. lobata*, and *H. pellucidula*, are estimated from short-term toxicity bioassays using the multifactor probit analysis (MPA) software on sublethal data. The sublethal effect is defined as 'net larva migration.' The 24, 48, 72, and 96-h EC50s (mg F^-/L) were 178.06, 63.90, 45.41, and 38.28 for *C. marginata*; 90.06, 36.20, 26.71, and 22.95 for *H. bulbifera*; 122.64, 42.45, 29.80, and 24.97 for *H. exocellata*; 238.50, 76.22, 52.12, and 43.09 for *H. lobata*; and 185.05, 53.74, 35.58, and 28.96 for *H. pellucidula*. SC values (or 8760-h EC0.01s) were 1.79 for *C. marginata*, 0.73 for *H. bulbifera*, 0.56 for *H. exocellata*, 1.18 for *H. lobata*, and 0.39 for *H. pellucidula*. Thus, *C. marginata* and *H. lobata* appear to be less sensitive species to fluoride toxicity during short-term and long-term exposures. This difference in sensitivity to fluoride among test species is not dependent upon the body size of net-spinning caddisfly larvae; Pearson correlation analysis between estimated SCs and larva dry weights was not significant ($P > 0.05$). On the other hand, SCs calculated for test species are lower than those proposed for other freshwater and marine animals. It is concluded that the multifactor probit analysis of sublethal acute toxicity data can be a valuable methodology in environmental toxicology to estimate accurate safe concentrations of chemical compounds for aquatic organisms.

Key words: Aquatic life; Fluoride toxicity; Safe concentrations.
Reprints: J A Camargo, Csic, Centro Ciencias Medioambientales, Serrano 115 Dpdo, E-28006 Madrid, Spain.

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8. **Discussion.** Deal with general conclusions, referring to other work on the subject. In short papers Results and Discussion may be combined.
9. **Abbreviations or Acronyms.** Define, either in brackets or in footnotes, when they first appear.
10. **Acknowledgements.** Keep brief. They may include funding source, technical assistance, text editing and useful comments.
11. **References.** See current issues of journal for usual style, which identifies references by superscripted numbers in the order in which references first occur. Other styles may be accepted, provided they are accurate and consistent.

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