President
Prof. A.K. Susheela
All India Institute of Medical Science
New Delhi, India

Second Vice-President
Ming-Ho Yu, Professor
Huxley College of Environmental Studies
Western Washington University
Bellingham, Washington, USA

Vice-President
H. Tsunoda, M.D., MD
Iwate Medical University
Morioka, Japan

Secretary
Prof. G.W. Miller, Ph.D.
Utah State University
Logan, Utah, USA

Treasurer
E.M. Waldott, B.A.
Warren, Michigan, USA

ADVISORY BOARD
Prof. Charles A. Baud, M.D., Ph.D.
Institute of Morphology
University Medical Center
Geneva, Switzerland

Prof. A.W. Burgeranter, Ph.D.
University of Kansas
Lawrence, Kansas, USA

K.R. Bulusu
Nagpur, India

Dr. G. Embery
Dept. of Dental Sciences
Univ. of Wales, Coll. of Med.
Cardiff, Wales, UK

Prof. J. Franke
Orthopedic Clinic
Medical Academy
Erfurt, GDR

Dr. Jean-Pierre Garrec,
Director, Laboratoire d'Etude
de la Pollution Atmospherique
Champeneux, France

Dr. C. James Lovelace
Department of Biology
Humbolt State University
Arcata, California, USA

Prof. G.W. Miller, Ph.D.
Utah State University
Logan, Utah, USA

Dr. Guy Milhaud
Service de Pharmacie et
Toxicologie, Ecole Nationale
Veterinaire d'Alfort
Maim-Alfort, France

Prof. J.B. Patrick, Ph.D.
Mary Baldwin College
Staunton, Virginia, USA

EDITORIAL BOARD
D.J. Ballantyne, Ph.D.
University of Victoria
Victoria, B.C., Canada

Dr. John A. Cooke
Sunderland Polytechnic School of
Pharmacy and Biology
Sunderland, England, UK

Dr. Edward Czerwinski, MD
Cracow Academy of Medicine
Krakow, Poland

Dr. Michael N. Eyed
Kimron Veterinary Institute
Beit Dagan, Israel

Prof. Jacques Elsair
Inst. des Sciences Medicales
Alger, Algeria

Prof. G. Neel Jenkins
Newcastle upon Tyne, England

Prof. J. Krechmik, Ph.D.
Director, Dept. of Toxicology
Akademia Medycyna
Gdansk, Poland

K.A.V.R. Krishnamachari, MD
National Institute of Nutrition
Hyderabad, India

Lennart Krook, DVM, Ph.D.
N.Y. State Coll. of Veterinary
Medicine, Cornell University
Ithaca, New York, USA

John R. Lee, MD
Mt. Valley, California, USA

Yu-Min Li, MD
Institute of Labor Protection
Changsha, China

Dr. Zygmun Machoy
Dept. of Biochemistry
Pomeranian Medical Academy
Szczecin, Poland

Dr. B.P. Raja, B.S., M.D.
Madras Dental College
Madras, India

Dr. Med. Hans Runge
Orthopedic Clinic
Martin Luther University
Halle, GDR

Prof. René Truhaft, Ph.D.
Faculté de Pharmacie
Université de Paris, France

H. Tsunoda, M.D., MD
Iwate Medical University
Morioka, Japan

Prof. Y. Yoshida
Oshaka Medical College
Osaka, Japan

Dr. F. Murray
School of Environmental and
Life Sciences, Murdoch Univ.
Murdoch, Western Australia

H.M. Sinclair, MD
Magdalen College
Oxford, England

Prof. A.K. Susheela
All India Inst. of Med. Sci.
New Delhi, India

Prof. S.P.S. Teotia, MD
Medical Coll., U. of Meerut
Meerut, India

Dr. Sally W. Wheeler
Hawkesbury Ag. Res. Unit
Richmond, N.S.W., Australia

Prof. Ming-Ho Yu
Huxley Coll. of Envir. Studies
Western Washington Univ.
Bellingham, WA, USA
FLUORIDE QUARTERLY REPORTS

Issued by
The International Society for Fluoride Research

TABLE OF CONTENTS

EDITORIAL

Dietary Fluoride Intake in the USA Revisited (Part 2) — by Robert Roy Kintner; Sioux Falls, South Dakota, USA .............. 51-61

ORIGINAL ARTICLES

Health Survey of Workers of an Aluminum Plant in China. I. Airborne Fluoride Levels in Work Environment and Bodily Fluoride Burden of Workers — by Humio Tsunoda, Kazuyoshi Itai, Shiro Sakurai, Fang-Ping Chen, Feng Liang, Ming-Ho Yu, Hirokazu Kudo, Shigenao Nakaya, Masanobu Tatsumi, Hui-Xian Ma, Chuan-Jie Mu, and Yu-Min Li; Morioka, Japan, Changsha and Tianjin, China, and Bellingham, Washington, USA 62-65

Health Survey of Workers of an Aluminum Plant in China. II. Study on Blood Chemistry — by Ming-Ho Yu, Hui-Xian Ma, Shiro Sakurai, Kazuyoshi Tsunoda, Kazuyoshi Itai, Masanobu Tatsumi, Shigenao Nakaya, Feng Liang, Fang-Ping Chen, Chuan-Jie Mu, and Yu-Min Li; Bellingham, Washington, USA, Tianjin and Changsha, China, Morioka, Japan ............. 66-70

The Quantitative Assessment of Bone Structure on the Radiograph in the Diagnosis of Fluorosis — by Edward Czerwinski; Krakow, Poland 71-75

Short-Term Toxicity of Fluoride Ion (F⁻) in Soft Water to Rainbow Trout (Salmo gairdneri) and Brown Trout (Salmo trutta Fario) — by J.A. Camargo and J.V. Tarazona; Madrid, Spain ......... 76-83

ABSTRACTS

(Corrected Abstract from October, 1990)

Fluctuation of Fluoride Concentrations in Drinking Waters: A Collaborative Study — by M.J. Larsen, O. Feyerskov, O. Bojen, F. Sunderovitz, D. Lambrau, F. Manji and M. Hobdell; Aarhus, Denmark, Godthaab, Greenland, Athens, Greece, Nairobi, Kenya, and Dublin, Ireland ....................... 84
The scientific program will consist of special lectures and keynote addresses which represent the areas of emphasis for the XIXth ISFR Conference presented by world experts. In addition, several workshops are being proposed that will represent some of the traditional areas of the Society's interest as well as those of growing interest. Workshops will be scheduled as simultaneous sessions and will consist of invited and contributed communications.

The official language of the conference will be English. No simultaneous translation will be available.

The conference will be held at the Renaissance Hall, and the banquets will be at Kyoto Century Hotel. Both facilities are located close to Japan Rail Kyoto Station.

The registration fee for active participants includes the cost of the welcoming reception, conference banquet, lunches, coffee breaks, a book of abstracts and tours of industrial operations. For social participants, the registration fee covers the cost of welcoming reception and conference banquet. Fees should be sent in Japanese currency only and drafts be made payable to the Osaka Medical College -- ISFR 92.

"I am convinced that the scientific program will be very interesting and rewarding for every participant. In addition to the scientific programs, we invite you to join tours of industrial operations involving fluorine containing chemicals such as hydrofluoric acid and fluoroorganic compounds. We look forward very much to welcoming you to Kyoto in September, 1992."

Y. Yoshida
Chairman, Organizing Committee

For further information, contact: ISFR 92 Secretariat, c/o Dr. Koichi Kono, Department of Hygiene and Public Health, Osaka Medical College, 2-7 Kaigakumachi, Takatsuki City, Osaka, Japan. TeleFAX 0726-84-6519.

FLUORIDE is published quarterly by the INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH, INC.

SUBSCRIPTIONS: $30.00 per annum in advance, including postage. $22.00 of the $25.00 Professional Membership fee is journal subscription. Researchers are invited to apply for membership.

MANUSCRIPTS for publication should be submitted in English, doublespaced with generous margins. References should be arranged according to the order in which they are cited in the text, and written as follows: Author, title, journal, volume, pages and year. Each paper must contain a summary ordinarily not exceeding 15 lines. Authors are also invited to include Key Words. Paper are accepted for publication after favorable evaluation and recommendation by qualified reviewers.

FLUORIDE is listed in: Current Contents/Agriculture, Biology & Environmental Sciences

COPIES of articles from this publication are now available from the UMI Articles Clearinghouse. Mail requests to University Microfilms International, 300 North Zeeb Road, Box 91, Ann Arbor, Michigan 48106, USA.
DIETARY FLUORIDE INTAKE IN THE USA REVISITED
Part 2

In the first section of this editorial the dietary fluoride (F⁻) intake of young adults (11-19 years) and adults (20-64 years) was discussed. The mean dietary F⁻ intake in low/unfluoridated communities was within the range proposed by water fluoridation proponents. Persons residing in fluoridated communities, on the other hand, were estimated to have mean F⁻ intakes up to three times the levels of the low F⁻ communities. Moreover, subgroups of the population having high fluid intakes were estimated to have total F⁻ intakes as high as 4.3 mg/day (young adults), 5.6 mg/day (adults), and 6.1 mg/day (adult males). This section of the editorial will consider the intake of infants (birth to 1 year of age) and toddlers (1-3 years of age).

Table 1 summarizes the results from a number of dietary F⁻ intake studies on the age groups of interest, presented chronologically by publication date.

**Baseline Studies**

A Canadian study prior to the introduction of fluoridation yielded a baseline value for dietary F⁻ intake (1). Analogous studies in the U.S. were not identified. Intake for 4-month-olds in low-F⁻ Stratford, Ontario (Canada) was 0.169 mg F⁻/day (0.026 mg F⁻/kg of body weight/day) whereas for one-year-olds intake was 0.564 mg F⁻/day (0.056 mg F⁻/kg body weight/day). The high baseline diet for the latter group was due to pablum in the diet, which because of its high bone meal content contributed 0.420 mg F⁻/day. Milk, a low F⁻ food diluted with water, provided the balance of the food portion of the diet. Formula was not a part of these diets. [The assumption may be made that infants, whose diet consisted mainly of breast milk (0.025 mg F⁻/L) or cow's milk (0.10 mg F⁻/L), had low F⁻ diets (8). Establishing a baseline for infants consuming formula or for toddlers would be more difficult due to lack of data.] While it was difficult to establish an F⁻ baseline intake for adults (pointed out in the first section of this editorial), the lack of pre-fluoridation studies makes it almost impossible to do so for toddlers and formula-consuming infants. The failure of proponents of community F⁻ programs to have done this critical research prior to the introduction of their programs attests to a lack of understanding of the full impact of F⁻ programs.

**Dietary Studies of Infant Intake**

Farkas and Farkas (2) estimated dietary F⁻ intake for a 6-month-old infant (formula and pablum cereal) in a fluoridated community at 1.95 mg F⁻/day (0.24 mg F⁻/kg/day).*

Watrowski and coworkers (3), using their F⁻ analysis values and dietary compositions recommended for infants from the Mayo Clinic Diet Manual, here and elsewhere in this editorial the values in ( ) are expressed in terms of mg F⁻/kg of body weight/day. Proponents of community fluoridation programs propose an infant intake of 0.05-0.07 mg F⁻/kg of body weight/day to achieve the maximum caries reduction benefits and consider an intake of about 0.1 mg F⁻/kg of body weight/day between birth and age 12 as capable of inducing dental fluorosis, one of the first signs of F⁻ toxicity (10). Induction of fluorosis has been suggested with an intake as low as 0.05 mg F⁻/kg of body weight/day (11) and susceptibility to be up to age 16 (7).
Table 1
Dietary Fluoride Intake for Infants and Toddlers

<table>
<thead>
<tr>
<th></th>
<th>Water ppm F⁻</th>
<th>Food &amp; Water mg F⁻/day</th>
<th>Beverage &amp; Water mg F⁻/day</th>
<th>Total mg F⁻/day (mg F⁻/kg/day)</th>
<th>Comment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3 Month Olds</td>
<td>1974</td>
<td>0.9</td>
<td>0.71</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79 (0.14)</td>
<td>Chicago</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>1.0</td>
<td>0.543</td>
<td>0.09</td>
<td>0.633 (0.13)</td>
<td>calc. for 2 mo.</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>0.1</td>
<td>0.041</td>
<td>0.009</td>
<td>0.050 (0.01)</td>
<td>calc. for 2 mo.</td>
</tr>
<tr>
<td>4-6 Month Olds</td>
<td>1950</td>
<td>0.16</td>
<td>0.133</td>
<td>0.036</td>
<td>0.169 (0.03)</td>
<td>Kitchener, Can (4 mo)</td>
</tr>
<tr>
<td></td>
<td>1950</td>
<td>1.29</td>
<td>0.170</td>
<td>0.289</td>
<td>0.459 (0.07)</td>
<td>Stratford, Can (4 mo)</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>1.0</td>
<td>1.69</td>
<td>0.26</td>
<td>1.95 (0.24)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>0.9</td>
<td>1.23</td>
<td>0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34 (0.17)</td>
<td>Chicago, 4-6 mo</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>1.0</td>
<td>0.62</td>
<td>0.143</td>
<td>0.763 (0.09)</td>
<td>calc. for 6 mo</td>
</tr>
<tr>
<td></td>
<td>1979</td>
<td>0.1</td>
<td>0.139</td>
<td>0.014</td>
<td>0.153 (0.02)</td>
<td>calc. for 6 mo</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>0.67</td>
<td>0.23</td>
<td>3.11</td>
<td>0.541 (0.07)</td>
<td>Orlando, 6 mo</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>1.04</td>
<td>0.115</td>
<td>0.092</td>
<td>0.207 (0.03)</td>
<td>Grand Rapids, 6 mo</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>0.60</td>
<td>0.15</td>
<td>0.122</td>
<td>0.272 (0.03)</td>
<td>Philadelphia, 6 mo</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>0.37</td>
<td>0.238</td>
<td>0.116</td>
<td>0.354 (0.04)</td>
<td>Los Angeles, 6 mo</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>&gt; 0.7</td>
<td>0.170</td>
<td>0.246</td>
<td>0.418 (0.05)</td>
<td>mean for 11 cities</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>0.3-0.7</td>
<td>0.166</td>
<td>0.148</td>
<td>0.314 (0.04)</td>
<td>mean for 6 cities</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>&lt; 0.3</td>
<td>0.179</td>
<td>0.047</td>
<td>0.226 (0.03)</td>
<td>mean for 5 cities</td>
</tr>
<tr>
<td>1 Year Olds</td>
<td>1950</td>
<td>0.16</td>
<td>0.492</td>
<td>0.072</td>
<td>0.564 (0.06)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Kitchener, Can</td>
</tr>
<tr>
<td></td>
<td>1950</td>
<td>1.29</td>
<td>0.545</td>
<td>0.587</td>
<td>1.123 (0.11)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Stratford, Can</td>
</tr>
<tr>
<td>2 Year Olds</td>
<td>1980</td>
<td>0.37</td>
<td>0.148</td>
<td>0.167</td>
<td>0.315 (0.03)</td>
<td>Los Angeles</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>0.65-1.04</td>
<td>0.147-0.197</td>
<td>0.252-0.407</td>
<td>0.410-0.610&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3 cities</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>&gt; 0.7</td>
<td>0.173</td>
<td>0.448</td>
<td>0.621 (0.05)</td>
<td>mean for 11 cities</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>0.3-0.7</td>
<td>0.141</td>
<td>0.245</td>
<td>0.386 (0.03)</td>
<td>mean for 6 cities</td>
</tr>
<tr>
<td></td>
<td>1985</td>
<td>&lt; 0.3</td>
<td>0.166</td>
<td>0.041</td>
<td>0.207 (0.02)</td>
<td>mean for 5 cities</td>
</tr>
</tbody>
</table>

Notes:
- Formula/milk are included in the food category. Fruit juices are included here when they cannot be identified separately.
- Computed assuming 0.9 ppm F⁻ water.
- The mean weight of one-year-olds was assumed to be 10 kg for this calculation.
- The ranges for total F⁻ are those reported by the author and are not the sums of the ranges shown for food and beverage/water.
arrived at the following infant intakes in a fluoridated community: 2- to 3-month-old, 0.78 mg F\(^{-}/\text{day}\) (0.14)*; and 4- to 6-month-old, 1.34 mg F\(^{-}/\text{day}\) (0.17). These infants' diets included fruit, vegetables and juice in addition to a homogenized-milk-product formula and cereal. Formula contributed 0.31 mg F\(^{-}/\text{day}\) to the 2- to 3-month-old child's diet and 0.37 mg F\(^{-}/\text{day}\) to that of the 4- to 6-month-old child's diet.

Maximum and minimum F\(^{-}\) dietary intakes for 2- and 6-month-old infants residing in fluoridated and unfluoridated areas according to Singer and Ophaug (4) were as follows for fluoridated areas (assuming they were consuming water at 1 ppm F\(^{-}\) and food that had been prepared and/or commercially processed with water at that level of F\(^{-}\)) and for unfluoridated areas (at 0.1 ppm F\(^{-}\)):

<table>
<thead>
<tr>
<th></th>
<th>1 ppm F(^{-}) water (mg F(^{-}/\text{kg/day}))</th>
<th>0.1 ppm F(^{-}) water (mg F(^{-}/\text{kg/day}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-month-olds</td>
<td>0.633 (0.13)</td>
<td>0.050 (0.010)</td>
</tr>
<tr>
<td>6-month-olds</td>
<td>0.763 (0.094)</td>
<td>0.150 (0.019)</td>
</tr>
</tbody>
</table>

For the higher F\(^{-}\) diets, the major F\(^{-}\) contributors were formula followed by water. The dramatically lower F\(^{-}\) content of the lower F\(^{-}\) diets was achieved by substituting human or cow's milk for formula and using low F\(^{-}\) water.

Using a regional market basket approach (described in the first section of this editorial), Singer's group produced three F\(^{-}\) dietary intake studies, one which included 6-month-old infants (5), one for two-year-old toddlers (6), and the third for 6-month- and 2-year-olds (7). Of the four cities included in the study of 6-month-olds, only Orlando (0.67 ppm F\(^{-}\)) with an intake of 0.541 mg F\(^{-}/\text{day}\) (0.07) had an intake in the range proposed for fluoridation programs (0.05-0.07 mg F\(^{-}/\text{kg of body weight/day}\)). Fluoridated Grand Rapids (1.04 ppm F\(^{-}\)) was established to have a lower intake [0.207 mg F\(^{-}/\text{day}\) (0.03)] than low F\(^{-}\) Los Angeles [(0.37 ppm F\(^{-}\)] at 0.34 mg F\(^{-}/\text{day}\) (0.04)]. Drinking water, dairy substitutes including formula, grain and cereal products, and vegetables were found to contribute between 65% and 86% of the F\(^{-}\) in diets constructed from market basket collections. Differences in amounts of water, formula, milk, and cereal/grain products assigned to a region's diet significantly influenced its F\(^{-}\) content. Fluoridated water and formula and cereal/grain products processed in fluoridated water elevated the F\(^{-}\) content of the diet (5).

In the second study according to these authors (6) (age 2) toddler intakes were lower than or similar to infant intakes in the same cities. This was attributed to lowered intake of ready-to-feed formula and dry infant cereals (high F\(^{-}\) if prepared with fluoridated water) and their replacement with other cereal and dairy products. When F\(^{-}\) intake was unchanged, it was attributed to increased toddler consumption of water and soft-drink-type beverages.

In their third study (7), this group estimated average intakes for the four dietary regions of the U.S. from a collection of 22 infant and toddler market basket collections spanning cities the municipal water F\(^{-}\) levels of which were 0.05 to 1.04 ppm F\(^{-}\). In this study total dietary F\(^{-}\) intake and community water F\(^{-}\) level for both infants and toddlers were well correlated.
However, food $F^-$ content failed to correlate with water $F^-$ level for either group. The higher the consumption of milk, the lower was the dietary $F^-$ intake for 6-month-olds. In southern cities dietary $F^-$ intake was higher than in north-central cities at the same water $F^-$ level, primarily because water intake in the south among infants was greater. This effect did not happen to toddlers. Water plus beverages were calculated to contribute up to 71.6% of the $F^-$ for diets of 2-year-olds who reside in high fluoride regions. Even though toddlers residing in fluoridated cities had significantly higher dietary $F^-$ intakes than infants, the intake per kilogram of body weight was almost identical (0.05 mg/kg of body weight/day) due to the higher weight of toddlers. These authors stressed that their determinations were for average diets in which it was assumed that both whole milk and formula were included rather than one or the other exclusively. Singer's group concluded that these average estimated diets did not exceed 0.08 mg $F^-$/kg of body weight/day in either infants or toddlers in any of the 22 cities studied. However, they suggested that toddlers brushing their teeth twice daily with $F^-$-containing dentifrice could be ingesting enough $F^-$ to nearly equal that received from the diet in a fluoridated city.

**Infant and Toddler Intake Reviews**

Singer and Ophaug (9) who summarized $F^-$ intakes of infants and toddlers noted that the $F^-$ content of infant dry cereals, fruit juices, and milk formulations were clearly raised when processed in $F^-$ water: cereals 3.0-10.1 times, fruit juices 5-50 times, and milk formulations 2.4-7.1 times. They also quoted studies that showed the $F^-$ content of beverages was about 10% less than the $F^-$ content of water used in their manufacture and pointed to the need to consider all sources of $F^-$ intake, especially that from dentifrice in small children whose swallowing reflex is poorly developed.

A recent World Health Organization (WHO) reported (8) estimated that $F^-$ intake of infants up to 6 months of age was as high as 0.20 mg/kg of body weight/day when water is fluoridated at 0.7-1.2 ppm; that the value for breast-fed infants was 0.003-0.004 mg/kg of body weight/day. For the 6- to 12-month-old child $F^-$ intake depended primarily on the proportion of tap water used to prepare infant food.

Rao (10) noted that $F^-$ content of infant formulas, toddler cereals, fruit juices and popular beverages increased significantly largely because fluoridated water was used in processing. This review also noted that childhood exposure to $F^-$ is significant when using $F^-$-containing dentifrices (0.4-1.2 mg $F^-$ from brushing and 0.2-0.4 mg $F^-$ after using mouthwashes, for 7-13-year-olds). Rao commented that diet, water and oral hygiene sources of $F^-$ intake must all be considered when assessing the total $F^-$ intake of children.

Ekstrand (11) noted that manufacturers failed to control the $F^-$ content of baby formulas prior to the 1980's to reduce infant $F^-$ exposure. Ekstrand recommended that manufacturers prepare their products using water with 0.15 mg $F^-$/L as an upper limit for ready-to-feed formula and when liquid concentrates or powdered formula is diluted with water in a fluoridated community to do so with bottled or deionized water to avoid too high an $F^-$ intake. He cited a study showing milk- and soy-based, ready-to-feed formulas, and liquid concentrate and powder formulas made with low $F^-$ water produced intakes from formula alone of between 0.021 and 0.054 mg $F^-$/kg of body...
weight/day for infants 1-6 months of age; liquid concentrates and powder formulas prepared with 1.0 ppm F⁻ water gave F⁻ intakes of 0.075-0.148 mg/kg of body weight/day. The milk-based formula delivered slightly less F⁻ than those that were soy-based and intake of a one-month-old exceeded that of a 6-month-old.

Identifying At-Risk Groups

Formula-Fed Infant Means: Infants primarily dependent upon formula appear to comprise one category at risk of F⁻ overdose. Table II lists calculated mean ranges of F⁻ dietary intakes for 1-, 3-, and 6-month-old infants using formula concentrates exclusively and prepared with 1 ppm F⁻ water. The assumptions and sources used in the estimation are noted in the table. As can be seen, the means all lie above the fluorosis threshold of 0.10 mg F⁻/kg of body weight/day. The F⁻ overdose of those with a high caloric intake would be still larger. With a reported caloric intake of 56.6% above the mean for the 0- to 6-month-old in the top 5th percentile (12), the corresponding F⁻ intake would be > 1.66-1.93 mg/day (> 0.21-0.24). Whereas 64% of infants are reported to use formula the first month of life, by the sixth month only 29% do so (13). Of non-nursing infants < 1 year, 24.3% used formula concentrates while 72.9% used ready-to-feed formula and 2.7% used both (12). In the 1970's diluted concentrate accounted for approximately 50% of sales; subsequently the fraction of powdered concentrate on the market has risen (12). Not the majority but a substantial number of infants consume formula concentrate the likely outcome of which is F⁻ intoxication during the time that the bulk of their nourishment is from formula. Were an adult (70 kg) to consume F⁻ at 0.15 or 0.24 mg/kg of body weight/day, it would be equivalent to daily consumption of 10.5 and 16.8 mg F⁻, respectively!

Table 2

<table>
<thead>
<tr>
<th>age mos.</th>
<th>wt. kg</th>
<th>vol. formula liters</th>
<th>milk/soy formula</th>
<th>milk/soy powder formula</th>
<th>other food</th>
<th>water &amp; beverages</th>
<th>total mg (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>0.7</td>
<td>0.47 (0.12)</td>
<td>0.59 (0.15)</td>
<td>0.1</td>
<td>0.47-0.59</td>
<td>(0.12-0.15)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.85</td>
<td>0.57 (0.10)</td>
<td>0.71 (0.12)</td>
<td>0.1</td>
<td>0.67-0.81</td>
<td>(0.11-0.14)</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>1.0</td>
<td>0.67 (0.08)</td>
<td>0.84 (0.10)</td>
<td>0.15</td>
<td>1.00-1.17</td>
<td>(0.13-0.15)</td>
</tr>
</tbody>
</table>

a Adapted from the data reported in Ekstrand’s Symposium Summary article (11).

b Food F⁻ contributed at age 6 months minus the 12.5% F⁻ contribution from milk and other dairy products and their substitutes for fluoridated cities reported by Ophaug and Singer (7).

c Contributions deduced from Ershow and Cantor’s Total Water and Tapwater Study (12) assuming processing/preparation/consumption of water and beverages at 1 ppm F⁻. The reported a total water intake for <1-year-old group of 1.148 L, of which 22.9% came from sources exclusive of foods, milk, and formula. The quantity of F⁻ from this source was reduced to 70% to more nearly reflect the intake of a 6-month-old infant.
It has been recommended that formula concentrate be diluted with bottled or deionized water to prevent F intoxication (11). The formula-dependent infant residing in a fluoridated community comprises one group ingesting toxic quantities of F. It should be stressed that the toxic level is reached at mean dietary consumption levels for this group. When delivered using water as a vehicle, the F dosage clearly cannot be kept below fluorosis levels without being defluoridated.

Total Water-Based F Intake Estimates

As pointed out in the earlier sections of this editorial, dietary surveys assessing F exposure determine the F values for the average diet constructed from composition studies or the mean for the groups studied. These studies have ascertained that F intakes of children are most often below or just at a fluorosis-producing level in fluoridated communities; however, they adequately identify neither the subgroups most at risk of overexposure nor the magnitude of any overexposure. For instance, in constructing an average diet for infants, milk (low F) and made-up formula concentrate (high F), the major food sources of infants, are combined. If a survey indicated milk consumption higher than that for formula in the population (assume formula 25%, milk 75%), the milk (negligible F content) will dilute the formula F concentration by 4 to which an exclusively formula-consuming infant would be exposed. This method of dietary assessment will virtually guarantee that overexposed groups will be difficult to identify.

The publication of Total Water and Tapwater Intake in the United States: Population-Based Estimates of Quantities and Sources by Ershow and Cantor (12), makes it possible to estimate not only mean F intakes based upon household diets, but also F intakes for those persons having fluid and caloric intakes in the higher percentiles of the population. This study which was used to compute intakes for mean and at-risk adults and young adults in Part I of this editorial, will now be used to do the same for infants (< 1 year of age) and toddlers (1- to 3-year-olds).*

The results for infants are shown in Table IIIA, those for toddlers in Table IIIB. For infants, especially those 6 months or younger, milk and milk- or soy-based formulas provide the major food and fluid intake. To identify those infants most at risk of high F exposure, likely intakes for a formula-fed infant were examined and included formula, juices, water and other beverages, all prepared, diluted, and/or processed with F water (1 ppm).

Infant F intakes in all categories are above the fluorosis intake level of 0.1 mg F/kg of body weight/day. The mean for the < 1-year-old group compared with that for the 6-month-old in Table II shows that it is lower, primarily due to decreased formula intake, a result of including the older infant (above 6 months and less than one year) who will rely less on formula. Nonetheless, the mean intake here also exceeds the fluorosis threshold 2-fold for the highest percentile. Since the survey upon which the estimates of Table IIIA are based did not include the liquid intake from nursing mothers (8.02% of infants), the formula and water intakes upon which these F estimates are based should be considered conservative (12). Since male and female infants

* It should be emphasized that the data from this study were collected in 1977-78; therefore, the values estimated here reflect consumption patterns of that period.
Table 3A
Water-Intake-Based Estimates of Daily Fluoride Intake
for Infants in Fluoridated Communities

<table>
<thead>
<tr>
<th>Group</th>
<th>wt, kg</th>
<th>formula</th>
<th>other</th>
<th>food</th>
<th>total (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>7.7</td>
<td>0.48</td>
<td>0.26</td>
<td>0.15</td>
<td>0.89 (0.12)</td>
</tr>
<tr>
<td>top 25%</td>
<td>7.7</td>
<td>&gt;0.57</td>
<td>&gt;0.31</td>
<td>0.15</td>
<td>&gt;1.03 (0.13)</td>
</tr>
<tr>
<td>top 5%</td>
<td>7.7</td>
<td>&gt;0.72</td>
<td>&gt;0.40</td>
<td>0.15</td>
<td>&gt;1.37 (0.16)</td>
</tr>
<tr>
<td>top 1%</td>
<td>7.7</td>
<td>&gt;0.91</td>
<td>&gt;0.47</td>
<td>0.15</td>
<td>&gt;1.53 (0.20)</td>
</tr>
</tbody>
</table>

a Milk and formula intake combined and assumed to be formula concentrate diluted with 1 ppm F⁻ water to yield diluted formula at 0.75 ppm F⁻. Higher percentile intakes scaled according to caloric intake for the age group (12).

b Value for water, juices and soft drinks assumed to be at 1 ppm F⁻. Higher percentile intakes adjusted by assuming intakes in these categories to be increased proportionally to that for total water intake for the age group (12).

c Using food F⁻ intake at 6 months minus 12.5% F⁻ intake contribution from milk and other dairy product substitutes in fluoridated cities (7). The F⁻ contribution of food was not scaled up to reflect increased caloric intake because the majority of calories were assumed to come from formula.

had similar liquid and caloric intakes, their F⁻ exposures were not considered separately.

The data for toddlers (1-3 years) in Table IIIB indicate mean F⁻ intakes for all toddlers and male toddlers to be just below the fluorosis level; however, all intakes above the upper 25th percentile exceed the fluorosis induction level, and those in the upper 5th percentile even when fluoridated dentifrice is excluded. The F⁻ exposure from dentifrice was included because toddlers are learning to brush their teeth at this age [over 90% of the dentifrice sold contains F⁻ (7) and in one English study 100% of youngsters had already begun to brush at least once a day by age 3 (cited in 17)] and may ingest substantial quantities of F⁻ because of poorly developed swallow reflexes (7,8,9,10 and references therein).

The F⁻ exposures in Table IIIB apply to the entire spectrum of toddlers since no special dietary assumptions were made. These values may be conservative because they do not include water intake from drinking fountains or from ice. Those in the highest percentile of intake risk an F⁻ exposure greater than twice that required to induce fluorosis.

At this point it is worth raising the question of why breast milk (as
## Water-Intake-Based Estimates of Daily Fluoride Intake for Toddlers in Fluoridated Communities

<table>
<thead>
<tr>
<th>Toddler Age Group</th>
<th>Food</th>
<th>Tap Water</th>
<th>Soft Drinks</th>
<th>Dentrifice</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both Sexes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.1</td>
<td>0.17</td>
<td>0.64</td>
<td>0.14</td>
<td>1.25</td>
</tr>
<tr>
<td>Top 25%</td>
<td>14.1</td>
<td>0.20</td>
<td>&gt;0.62</td>
<td>&gt;0.16</td>
<td>&gt;1.48</td>
</tr>
<tr>
<td>Top 5%</td>
<td>14.1</td>
<td>0.26</td>
<td>&gt;1.42</td>
<td>&gt;0.22</td>
<td>&gt;2.20</td>
</tr>
<tr>
<td>Top 1%</td>
<td>14.1</td>
<td>0.32</td>
<td>&gt;1.90</td>
<td>&gt;0.27</td>
<td>&gt;2.79</td>
</tr>
<tr>
<td>Males Only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.4</td>
<td>0.18</td>
<td>0.68</td>
<td>0.14</td>
<td>1.30</td>
</tr>
<tr>
<td>Top 25%</td>
<td>14.4</td>
<td>0.21</td>
<td>&gt;0.87</td>
<td>&gt;0.17</td>
<td>&gt;1.55</td>
</tr>
<tr>
<td>Top 5%</td>
<td>14.4</td>
<td>0.30</td>
<td>&gt;1.48</td>
<td>&gt;0.23</td>
<td>&gt;2.29</td>
</tr>
<tr>
<td>Top 1%</td>
<td>14.4</td>
<td>0.35</td>
<td>&gt;2.06</td>
<td>&gt;0.28</td>
<td>&gt;2.99</td>
</tr>
</tbody>
</table>

a The mean for the food portion of the diet is the value for the average diet taken from Ophaug and Singer (7) for toddlers (2-year-olds) and increased for the higher caloric consumption of the higher percentiles using caloric intake data from Ershow and Cantor (12).

b Includes 1 ppm F⁻ drinking water and water used in the home for the preparation of food and beverages. Excludes water in food and ready-to-drink beverages as purchased (12).

c The soft drink intake was calculated as 10% of the total water intake (12) and assumed to be 1 ppm F⁻.

d Estimated assuming a small child using fluoridated dentifrice (7-10 and references therein). Inclusion of this contribution probably does not apply until about age two or after for most children.

Evidence of Toxic F⁻ Intakes

Since infant and toddler F⁻ intakes frequently exceed the intoxication level for a substantial fraction of the population, the anticipated increased fluorosis in the permanent teeth some 7-15 years later (11) has been recorded (14-17). In one study more than one-half of those aged 6-12 exhibited detectable dental fluorosis in a fluoridated city, whereas 12.2% were detected in an unfluoridated community. The value for the unfluoridated city was the same as that for a naturally fluoridated city prior to implementation of community fluoridation (16). Current fluorosis values are several-fold higher than...
reported for cities of similar F⁻ water levels in the 1940's before initiation of artificial fluoridation (16). Because of the delay between F⁻ exposure and permanent tooth eruption and examination, current fluorosis levels reflect F⁻ exposures more than a decade ago.

Smith (18) pointed out that increased F⁻ intake from a fluoridated community raised the mean blood ionic F⁻ steady state concentration for the total population. Individuals having a higher blood F level are at greater risk of having peak blood F⁻ concentrations which exceed the threshold levels for damage to sensitive cells. It is wishful thinking to assume that only tooth-forming cells are adversely affected by high blood F⁻ peak levels. Smith also notes that fluorosis must be recognized for what it is: an irreversible pathological condition which is universally recognized as the first sign of systemic chronic F⁻ poisoning. A currently rising fluorosis level is alarming in that it reflects exposure in the 1970's. The results of current levels of exposure are yet in the future.

In recent years sources of F⁻ exposure such as fluoridated rinses, gels, mouth washes, supplement tablets, dental treatments, and tooth paste have proliferated because of commercial and professional endorsements. Since these sources were not factors when water fluoridation was instituted, total F⁻ exposure is most certainly higher than that anticipated at the outset.

Fluoride intake is poorly controlled in water fluoridation programs. As a result, a wide range of dosages are administered enhancing the possibility of F⁻ overdose. Data recently reported by Clovis and Hargreaves (19) illustrates this lack of control. Their study which included only beverage consumption by 6th graders (12-year-olds), was based upon 3-day-drink-diary records, and represented beverages actually consumed. Carbonated soft drinks were bottled in the fluoridated community but sold in both towns. The volume and corresponding F intakes for the three students consuming the highest and lowest beverage amounts for each community are given below.

<table>
<thead>
<tr>
<th>3 Highest 3-Day Intakes</th>
<th>3 Lowest 3-Day Intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/day mg/day</td>
<td>L/day mg/day</td>
</tr>
<tr>
<td>Unfluoridated Community:</td>
<td></td>
</tr>
<tr>
<td>2.87-3.38 0.53-0.82 (1.6-fold)</td>
<td>0.32-0.52 0.02-0.35 (17.5-fold)</td>
</tr>
<tr>
<td>Fluoridated Community:</td>
<td></td>
</tr>
<tr>
<td>2.12-2.77 0.95-2.45 (2.6-fold)</td>
<td>0.49-0.60 0.40-0.89 (2.3-fold)</td>
</tr>
</tbody>
</table>

The wide ranges of F⁻ intake within the same category reflect differences in beverage selection. Note the large differences in volume of beverage consumption, < 1/3 L to > 3 L, and the difference in F⁻ dosage, not only within groups with similar volume of consumption, but also within the same community [unfluoridated: 0.02-0.82 mg (41-fold) : fluoridated: 0.4-2.45 mg (6.1-fold)].
Conclusion

Gray (20) noted that a decline in caries has occurred in both fluoridated and unfluoridated areas. The decline has been noted elsewhere (21,22 and references therein). Gray pointed out that residents of British Columbia, where only 11% consume F⁻ water, have a lower caries rate than residents of provinces where 40-70% drink F⁻ water. Additionally, he notes that F⁻ containing tooth paste is normally discounted when water fluoridation is discussed, and he recognizes the negative effect of the social upheaval which usually accompanies a water fluoridation referendum. In view of these points, he calls for the recognition of changing times with regard to evaluating water fluoridation programs.

The evidence put forth in this editorial strongly argues for the discontinuation of water fluoridation programs. To summarize, such action is supported because:

1. There is a high likelihood that substantial portions of the young adult and adult populations (addressed in Part i of this editorial), as well as the infant and toddler populations, especially those with high fluid intake, are being exposed to F⁻ levels capable of producing cell damage (of which fluorosis is the outstanding manifestation).

2. A proliferation of F⁻-containing products that are now available contributes in an unplanned manner to the total F⁻ intake.

3. Exposure to F⁻ is uncontrolled because normally individuals select the volume and components of their diets in disregard to its F⁻ content.

4. The current impact of water fluoridation on caries is questionable due to an essentially equal or greater caries decline in unfluoridated communities.

References


**********

Robert Roy Kintner, Ph.D.
Chemistry Department
Augustana College
Sioux Falls, SD 57197
HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN CHINA

I. Airborne Fluoride Levels in Work Environment and Body Fluoride Burden of Workers

by

Humio Tsunoda,1 Kazuyoshi Itai,1 Shiro Sakurai,1 Fang-Ping Chen,2 Feng Liang,2 Ming-Ho Yu,2 Hirokazu Kudo,1 Shigenao Nakaya,1 Masanobu Tatsumi,1 Hui-Xian Ma,4 Chuan-Jie Mu,4 and Yu-Min Li4
Morioka, Japan; Changsha and Tianjin, China; Bellingham, Washington, USA

SUMMARY: The airborne fluoride levels in the work environment of an aluminum plant in China have been studied, and the data were correlated with the body fluoride burden of production workers and office workers (controls). Average gaseous and particulate F levels of the work environment were 0.65 mg/m³ and 0.21 mg/m³, respectively. The serum and urinary F levels of the exposed group were more than twice as high as those of the controls.

KEY WORDS: Airborne F; Aluminum plant; Body F burden; Occupational health survey.

Introduction

A high prevalence of endemic fluorosis exists in China. According to Wang et al. (1), 40 million persons out of a total population of 1.1 billion are afflicted with dental and/or skeletal fluorosis. The nature of endemic fluorosis in China varies with geographical regions. In the northern regions, fluorosis appears to be caused mostly by high levels of F in drinking water, whereas in the southwestern regions foodborne fluoride appears to be responsible for the disease (2,3).

A large number of reports have been published concerning serum F or urinary F levels in subjects exposed to environmental fluoride in different countries (4-10). Published data strongly suggest that, due to the fluoride derived from their living environment, the body burden of many Chinese may be higher than that of populations in the developed countries. Similarly, Chinese workers occupationally exposed to fluoride have higher body F burden than workers in industrialized countries. In the U.S., Germany, and Japan, the exposure limit of fluoride in the workplace is 2.5 mg/m³, whereas in China it is 1.0 mg/m³, and in Yugoslavia, the Maximum Acceptable Concentration (MAC) is 1.7 mg/m³ (10).

Through the collaboration of scientists from China, Japan, and the U.S., a health survey of workers employed at an aluminum plant in China was

1 Iwate Medical University, Morioka 020 Japan.
2 Institute of Labor Protection, CNNC, Changsha, China.
3 Western Washington University, Bellingham, WA 98225, USA.
4 Institute of Radiation Medicine, Chinese Academy of Medical Science, Tianjin, 300192 China.


62
conducted in 1987. Fluoride levels in the work environment were determined and the data were correlated with the health status of the subjects based on environmental health, biochemical, and clinical point of view. Fluoride levels in the work environment have been shown to be slightly below the Threshold Limit Value (TLV) of ACGIH. In this paper, the first of a series of four, we report a correlation study on the airborne F levels in the workplace with the serum and urinary F levels of workers living in an area with high levels of foodborne F.

Materials and Methods

For the study of body F burden, a total of 149 plant workers were chosen. The subjects were divided into two groups: potroom workers (n = 101), and office workers (n = 48) who served as controls. Body burden of fluoride was estimated by analysis of serum and urinary fluoride levels. Urinary samples were collected from potroom workers for the entire six working hours, whereas 24 hour samples were collected from some of the control group. Fluoride content was determined by the fluoride ion specific electrode method. Blood samples were taken at the time of physical examination and were analyzed by flow injection using the fluoride ion specific electrode method.

For determination of airborne F levels, 20 air samples were collected from different sites of the potroom, with a respirable personal dust sampler, model PS-45 (Manuf. Sibata, Tokyo). The filter holder of the sampler was modified to include a two-step filter system composed of alkali-treated and membrane filter paper so that both gaseous and particulate forms of fluoride could be collected. The sampler was run for one hour each time, at a flow rate of one liter/minute. Gaseous F collected by the alkali-treated filter paper and the soluble particulate F collected by the membrane filter paper were eluted with deionized water. The insoluble particulate F remaining on the membrane filter paper was extracted following separation by pyrohydrolysis apparatus. The extracted fluoride was then determined by flow injection analysis using the fluoride ion specific electrode.

Results and Discussion

As shown in Table 1, average total F levels in the air of the workplace of the plant were 0.89 mg/m³, with a range of 0.23-3.00 mg/m³. Gaseous F accounts for 0.65 mg/m³ (73%), with a maximum value of 2.4 mg/m³. On the other hand, soluble particulate form of fluoride was 0.21 mg/m³, with a maximum value of 1.2 mg/m³, while the concentration of insoluble particulate F was very low. Saric et al. (10) reported that the concentrations of HF in different working places ranged from 0.27 to 4.1 mg/m³, and particulate fluoride ranged from 0.02 to 1.6 mg/m³, while Wergeland et al. (11) in 1987 showed monthly averages of 2-4 mg/m³ F. Most of the observed values are below the Threshold Limit Value of ACGIH (HF: 2 mg/m³; fluoride: 2.5 mg/m³). The respirable dust levels in the air were 2.43-24.7 mg/m³.

Serum F levels of the exposed groups (0.055 ppm) were more than twice as high as the control values (0.025 ppm) (Table 2). Similarly, the urinary F concentrations of the exposed groups (2.99 ppm) were about 2.3 times higher than the controls (1.33 ppm). No significant differences were found in either the serum F levels or urine F levels among the four exposed groups (Groups A through D), although the length of their employment at the plant differed from each other (Table 2).
Table 1
Airborne Fluoride Levels in the Workplace

<table>
<thead>
<tr>
<th>Form of Fluoride</th>
<th>Concentration (mg F/m^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaseous</td>
<td>0.654 ±0.614 (0.098-2.42)</td>
</tr>
<tr>
<td>Particulate</td>
<td></td>
</tr>
<tr>
<td>Soluble</td>
<td>0.207 ±0.250 (0.037-1.20)</td>
</tr>
<tr>
<td>Insoluble</td>
<td>0.027 ±0.028 (0.004-0.097)</td>
</tr>
<tr>
<td>Total Fluoride</td>
<td>0.888 ±0.753 (0.23-3.00)</td>
</tr>
</tbody>
</table>

Values are means ±S.D. Values in parentheses indicate ranges.

Table 2
Serum and Urinary Fluoride Levels of Aluminum Plant Workers

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure Age</th>
<th>Serum F (mg/L)</th>
<th>Urinary F (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time (yr)</td>
<td>(mg/L)</td>
<td>(mg/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mean ±S.D.)</td>
<td>(mean ±S.D.)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>25-50</td>
<td>0.025 ±0.012</td>
</tr>
<tr>
<td>(n = 48)</td>
<td></td>
<td>(0.008-0.061)</td>
<td>(0.38-3.80)</td>
</tr>
<tr>
<td>Exposed</td>
<td>5-9</td>
<td>25-29</td>
<td>0.053 ±0.046</td>
</tr>
<tr>
<td>(n = 31)</td>
<td></td>
<td>(0.017-0.210)</td>
<td>(0.71-7.20)</td>
</tr>
<tr>
<td>A</td>
<td>10-19</td>
<td>30-34</td>
<td>0.053 ±0.024</td>
</tr>
<tr>
<td>(n = 22)</td>
<td></td>
<td>(0.020-0.096)</td>
<td>(1.60-11.80)</td>
</tr>
<tr>
<td>C</td>
<td>10-19</td>
<td>35-39</td>
<td>0.049 ±0.019</td>
</tr>
<tr>
<td>(n = 19)</td>
<td></td>
<td>(0.019-0.090)</td>
<td>(0.90-5.20)</td>
</tr>
<tr>
<td>D</td>
<td>&gt;20</td>
<td>&gt;40</td>
<td>0.061 ±0.064</td>
</tr>
<tr>
<td>(n = 29)</td>
<td></td>
<td>(0.029-0.379)</td>
<td>(0.69-11.20)</td>
</tr>
</tbody>
</table>

Values are means ±S.D. Values in parentheses indicate ranges.

The 24-hour excretion of F by 16 individuals in the control group was 1.22 ±0.63 mg (mean ±S.D.) while the concentration was 0.93 ±0.39 ppm. This value is 1.7 times higher than the reference value of 0.78 mg/day or 0.54 ppm for Japanese male adults of the same age group. Thus, it is estimated that the body F burden of these inhabitants may be 1.7 times as high as the Japanese value. Based on the average urinary F levels, the daily F intake of an adult Japanese male has been estimated at 1.56 mg. By use of the same method, the total F intake of the inhabitants of this region was calculated, giving 2.44 mg per day. The urinary and serum F levels of the potroom workers were more than twice as high as those of the control. Based on this, the total F intake of the refinery workers in this region is estimated at about 5 mg per day, and one-half of this amount is derived from the airborne F in the workplace. Elevated urinary F concentrations in children living in an area adjacent to an aluminum plant in Poland have been reported to be associated with increased F levels in the atmosphere and diet (8).
In the U.S., it has been shown that no osteofluorosis occurred among workers engaged in a workplace where the airborne F levels were below 2.5 mg/m³ and their urinary F levels do not exceed 5 mg/L. Furthermore, it is stated that workers engaged in such a work environment should not develop lung disease, kidney malfunction, or skin disorders (12).

Results of this survey have indicated that the airborne F levels in the work environment of the refinery were mostly below 2.5 mg/m³. However, although average urinary F levels of the potroom workers did not exceed 5 mg/L, 3.0% of these subjects exhibited concentrations in excess of 9 mg/L, a level that could possibly cause osteofluorosis. In other words, the urinary F levels of the subjects were high despite the fact that the airborne F levels in the workplace were low. This is attributed to the relatively high levels of fluoride in their living environment. Wei et al. (3) reported that the total F intake by subjects suffering from endemic foodborne fluorosis in Guizhou was as high as 8.6 mg/day. Based on these observations, it is conceivable that the workers engaged in this refinery plant may not have such a high body F burden as to cause skeletal fluorosis.

Acknowledgement

We thank the China National Nonferrous Metals Industry Corporation for support during the course of this study.

References


**********
HEALTH SURVEY OF WORKERS OF AN ALUMINUM PLANT IN CHINA

II. Study on Blood Chemistry

by

Ming-Ho Yu,¹ Hui-Xian Ma,² Shiro Sakurai,³ Humio Tsunoda,² Kazuyoshi Itai,³ Mananobu Tatsumi,³ Shigenao Nakaya,³ Feng Liang,⁴ Fang-Ping Chen,⁴ Chuan-Jie Mu,⁴ and Yu-Min Li²

Bellingham, Washington, USA; Tianjin and Changsha, China; Morioka, Japan

SUMMARY: Blood samples obtained from 96 production line workers supposedly exposed to airborne fluoride at an aluminum plant in China have been studied and the results compared with those obtained from 47 office workers (controls). The levels of creatinine were found to be significantly decreased, whereas the activity of leucine aminopeptidase increased, in the F-exposed group. Exposure to F did not appear to significantly affect the levels of hematocrit, total cholesterol, and BUN, or the activity of serum GOT, GPT, ALP, and LDH of plant workers.

KEY WORDS: Aluminum plant workers; Blood chemistry; Creatinine; Leucine aminopeptidase

Introduction

Part of a health survey of workers at an aluminum plant in China has been reported (1). Based on serum and urinary F levels, we estimated the daily total F intakes of the potroom workers and office workers (control) to be about 5 mg and 2.5 mg, respectively, and that these values were about 1.7 times greater than those commonly found among Japanese subjects. Blood samples obtained from the workers under study have been subjected to blood chemistry analysis in an attempt to correlate the results with fluoride exposure.

Materials and Methods

Participants in this study consisted of 96 production line workers (F-exposed) and 47 office workers (control) in an aluminum plant located in the southwestern part of China. They were first divided into three sub-groups according to their age, i.e., 25-34, 35-44, and 45-54 years of age. The first F-exposed group (25-34 years old) was further divided into two sub-groups based on employment time or F exposure time: those exposed for 5-9 years and 10-14 years, respectively (Table 1).

Blood samples were taken during the physical examination at a hospital.

¹ Western Washington University, Bellingham, WA 98225, USA.
² Institute of Radiation Medicine, Chinese Academy of Medical Sciences, Tianjin, 300192 China.
³ Iwate Medical University, Morioka 020 Japan.
⁴ Institute of Labor Protection, CNNC, Changsha, China.

Presented at the 17th ISFR Conference in Budapest, Hungary, June 22-25, 1989
Serum samples were analyzed in an automatic blood analyzer (Technicon, model SMAC III) for total protein, albumin, globulin, creatinine, and blood urea nitrogen (BUN), and the activity of glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), whereas hematocrit and total cholesterol were determined by conventional methods.

Data were analyzed either by the Student's t-test or by the method of Welch, followed by the t-test.

**Results**

The results of the blood chemistry analysis are shown in Table 1. Except for the creatinine and LAP, no significant differences in any of the parameters studied were observed between the control and the F-exposed groups, or among the sub-groups. Age was not found to affect any of the parameters studied. Among the F-exposed sub-groups, blood chemistry was found to be unaffected by exposure time.

A comparative study has been found on the activities of GOT, GPT, ALP, LDH, and LAP between the control and the F-exposed groups. The activity of each of the enzymes shown by the control group was considered as 100, and the relative value for the F-exposed group was calculated, and the results are shown in Figure 1. It is clear that, among the enzymes tested, significant changes occurred only in LAP. Leucine aminopeptidase was increased significantly in two sub-groups of the F-exposed subjects (Table 1). The increase was diminished with increase in age and exposure time. For the age groups 25-34 years, for example, the increase was 24% and 19% (p < 0.01) for those exposed for 5-9 years and 10-14 years, respectively. Differences between the 35-44 year-old control and the F-exposed group, however, decreased to 11% (Figure 1).

The levels of creatinine in the subjects exposed to F or 15 years and above were significantly (p < 0.01) decreased (Table 1). For the subjects exposed to 15 years, the decrease was 40%, whereas for those exposed to 20 years or more it was 31%.

The number of cases with abnormal clinical readings are shown in Table 2. It should be noted that abnormal cases for total protein refer to levels lower than normal values, whereas for GOT, GPT, ALP, LAP, and BUN, the subjects manifested an elevated value.

**Discussion**

A large number of reports have been published concerning the biochemical effects of fluoride on liver, kidney, or bone-forming cells in humans or experimental animals. Ferguson and Stephen (2) administered 1 mg F daily to 13 subjects residing in a non-fluoridated area and observed an initial decline in plasma alkaline phosphatase levels. Studying endemic fluorosis, Jeji et al. (3) reported a significant elevation in the levels of Serum alkaline phosphatase and glutathione. Kessabi et al. (4) also reported increased levels of alkaline phosphatase in sheep from an endemic fluorosis area in Morocco. In a study of the activities of various enzymes in the liver and kidney of mice exposed to fluoride, Singh (5) reported that alkaline phosphatase activity decreased.

Fluoride
## Table 1
Blood Chemistry of Aluminum Plant Workers

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Exposure time (yr)</th>
<th>N</th>
<th>HT %</th>
<th>GOT u/L</th>
<th>GPT u/L</th>
<th>ALP u/L</th>
<th>LDH u/L</th>
<th>T-Ch. mg/dL</th>
<th>LAP u/L</th>
<th>BUN mg/dL</th>
<th>Creatinine mg/dL</th>
<th>T-Pro. g/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-34</td>
<td>0</td>
<td>18</td>
<td>39.7±4.0</td>
<td>17.0±4.3</td>
<td>12.6±5.2</td>
<td>144±35</td>
<td>33.7±11.0</td>
<td>165±31</td>
<td>176±30</td>
<td>15.1±5.2</td>
<td>0.82±0.25</td>
<td>8.1±0.7</td>
</tr>
<tr>
<td>5-9</td>
<td>27</td>
<td>27</td>
<td>40.8±3.2</td>
<td>18.7±4.1</td>
<td>12.4±5.9</td>
<td>136±80</td>
<td>36.0±11.1</td>
<td>164±38</td>
<td>218±46**</td>
<td>13.6±3.0</td>
<td>0.71±0.24</td>
<td>8.1±1.0</td>
</tr>
<tr>
<td>10-</td>
<td>24</td>
<td>18</td>
<td>10.1±5.4</td>
<td>12.2±5.9</td>
<td>140±42</td>
<td>39.4±9.1</td>
<td>167±26</td>
<td>210±41**</td>
<td>16.3±4.7</td>
<td>0.69±0.19</td>
<td>8.3±1.1</td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>0</td>
<td>14</td>
<td>41.1±3.5</td>
<td>16.9±5.3</td>
<td>10.7±4.8</td>
<td>153±81</td>
<td>40.5±11.1</td>
<td>180±24</td>
<td>181±15</td>
<td>15.1±3.0</td>
<td>1.14±0.29</td>
<td>8.3±1.0</td>
</tr>
<tr>
<td>45-54</td>
<td>0</td>
<td>15</td>
<td>39.3±3.8</td>
<td>17.4±5.3</td>
<td>11.6±5.5</td>
<td>138±49</td>
<td>35.1±12.3</td>
<td>173±27</td>
<td>201±35*</td>
<td>13.9±5.3</td>
<td>0.68±0.23</td>
<td>6.9±0.7</td>
</tr>
<tr>
<td>20-</td>
<td>28</td>
<td>30.5±3.6</td>
<td>19.5±5.9</td>
<td>10.4±3.6</td>
<td>151±80</td>
<td>43.1±13.0</td>
<td>181±30</td>
<td>192±45</td>
<td>15.1±4.7</td>
<td>0.76±0.21**</td>
<td>7.8±0.8</td>
<td></td>
</tr>
</tbody>
</table>

*0 year exposure refers to office workers (control).

**Means ± S.D.

u/L, unit per liter; T-Ch., total cholesterol; T-Pro., total protein

\* p < 0.05; ** p < 0.01

## Table 2
Aluminum Plant Workers with Abnormal Clinical Readings

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Exposure time (yr)</th>
<th>n</th>
<th>GOT case</th>
<th>%</th>
<th>GPT case</th>
<th>%</th>
<th>ALP case</th>
<th>%</th>
<th>LAP case</th>
<th>%</th>
<th>BUN case</th>
<th>%</th>
<th>T-Pro. case</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-34</td>
<td>0</td>
<td>19</td>
<td>3.6</td>
<td>2</td>
<td>7.1</td>
<td>1</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
<td>5.3</td>
<td></td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>5-9</td>
<td>28</td>
<td>1</td>
<td>3.6</td>
<td>2</td>
<td>7.1</td>
<td>1</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
<td>5.3</td>
<td></td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>10-</td>
<td>24</td>
<td>1</td>
<td>4.2</td>
<td>1</td>
<td>4.2</td>
<td>1</td>
<td>3</td>
<td></td>
<td>12.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-</td>
<td>18</td>
<td>2</td>
<td>11.1</td>
<td>1</td>
<td>5.6</td>
<td>8</td>
<td>44.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>15</td>
<td>2</td>
<td>13.3</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>6.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-</td>
<td>28</td>
<td>1</td>
<td>3.6</td>
<td>2</td>
<td>7.1</td>
<td>1</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*0 year exposure refers to office workers (control)

Normal values for GOT and GPT are greater than or equal to 5 or less than or equal to 30; ALP: greater than or equal to 30 or less than or equal to 50; LAP: greater than or equal to 50 or less than or equal to 90; BUN: greater than or equal to 10 or less than or equal to 30; T-Pro.: greater than or equal to 8.7 or less than or equal to 8.3.
in the liver but increased in the kidney. Farley et al. (6) reported that NaF can increase the proliferation rate of bone cells and increase the alkaline phosphate content of bone cells and of embryonic bone. In the present study, no significant changes in alkaline phosphatase activity were observed between the control and the F-exposed groups (Table 1). This may indicate that exposure to the existing working environment may not contribute to significant changes in the skeletal system of the workers.

A marked increase in the activity of LAP was observed in the F-exposed group (Table 1; Figure 1). The enzyme is widely distributed in the body, such as in the brain, kidneys, liver, and pancreas. A possible leakage of the enzyme can occur following injury of any of these organs. The observed increase in LAP activity suggests F-induced injury of the liver or the pancreas (7).

Hematologic changes in patients with fluorosis have been reported. Guminska and Sterkowicz (8) showed a significant decrease in erythrocyte ATP levels in subjects chronically exposed to fluorides emitted from an aluminum plant in Poland. The decrease was attributed to fluoride-induced inhibition of glycolysis. A non-significant but consistent decrease in blood glucose level, together with an increase in lactate level, was also observed. Subsequently, Guminska et al. (9) reported decreases in erythrocyte and urinary magnesium levels in human subjects chronically exposed to environmental fluorides. Administration of magnesium, either alone or in combination with calcium or potassium, restored the magnesium levels. Guminska et al. (10), furthermore, observed elevated blood lactate and glucose and decreased ATP levels in individuals exposed to environmental fluorides. These changes also were reversed by administration of magnesium salts.
Separating specific effects of fluoride from other factors, such as nutritional factors, is thought to be difficult (11). In this study, changes in hematocrit readings were observed, but the differences were insignificant (Table I).

Acknowledgement

We thank the China National Nonferrous Metals Industry Corporation for support during the course of this study.

References

THE QUANTITATIVE ASSESSMENT OF BONE STRUCTURE ON THE RADIOGRAPH IN THE DIAGNOSIS OF FLUOROSIS

by

Edward Czerwinski
Krakow, Poland

SUMMARY: Radiograph microdensitometric measurements, using original computer programs, were performed in a group of veteran aluminum workers diagnosed by clinical and radiological criteria as having fluorosis. The workers' ages averaged 49.3 years, and they had been employed in the aluminum refinery for an average 19.7 years. The microdensitometric analysis produced quantitative measurements of bone structure including trabecular number, trabecular width, and density. Trabecular width increased proportionately with work exposure duration, especially in subjects with stage I fluorosis. This method can be effectively applied in the diagnosis of the early stages of fluorosis for which conventional radiography is inadequate.

KEY WORDS: Aluminum workers; Bone structure; Fluorosis; Microdensitometry; Osteofluorosis; Poland.

Introduction

Typically the diagnosis of osteofluorosis depends on skeletal radiographic changes. Roholm described the first phase as follows: "the density of bone is little increased, the trabeculae are rough, blurred and give deep shadows." In the more advanced stages, "trabeculae merge together and bone has a marble-like appearance." Due to improved industrial techniques that have resulted in markedly reduced worker fluoride exposure, the advanced stages are now rarely seen. Typical cases fall into the prestages or fluorosis "O" or "Ol" (1,2). Diagnosis of these early stages by conventional radiography is questionable even by those informed concerning the subject's fluoride exposure (3,4). An objective method of bone structure analysis using radiographs for the purpose of aiding in the diagnosis of fluorosis is herewith presented.

Materials and Methods

A single radiograph of the right supinated forearm was taken in the AP view with a calibrated stepped wedge (1-20 mm) of aluminum placed along the limb during exposure. Bone structure was assessed at the distal radial metaphysis and classified as normal, thickened, or fine. Osteosclerosis was classified in degrees from 1-3 according to changes observed.

Objective bone structure assessment of the radiograph was based on microdensitometric measurements using a standard Carl Zeiss, Jena, microdensitometer. Measurements were done automatically on a projected line on the metaphysis perpendicular to the long axis of the forearm. Also recorded was...
the radiographic darkening of the aluminum wedge steps. These results were entered into the computer and processed by several of the author's programs (5). The first program identified the bone borders and selected 128 points in the bone center. The density of each point was then derived from the calibration curve generated for the film from the wedge measurements. Generated this way, a microdensitometric curve representing the cross section of the bone structure was obtained. Using the geometric definition of the trabeculae as the basis of its algorithm, an automatic program distinguished the starting and ending points of the trabeculae. From this it was possible to calculate the numerical characteristics of the bone structure patterns as: number of trabeculae in the scan, their width, density, height, and area. The height was expressed as the percentage of the span between the minimum and maximum measurements; and area of trabeculae as the percentage of the total area of the scan (5,6) (Figure 1).

All data from the standard evaluation of the radiograph, the microdensitometric analysis, and the case history were entered into the computer and processed. According to the variable character Student t-test, the correlation coefficient or multiple Duynan's test were used in verifying the hypotheses (7).

Results

In 20% of the subjects, various alterations of bone structure were observed by unaided assessment of the radiographs (Table 1, Figure 1). Trabecular thickening was the most characteristic feature of this group. Osteosclerosis was infrequent, and cases of the 3rd degree were rare (3.6%). Pre-stages of fluorosis were relatively numerous (44.6%), due to criteria of subject selection.

Every analysis of the microdensitometric curve was presented on the plot and verified to eliminate possible errors. No false recognition of the trabeculae was found in this series. Analysis of different types of bone structure is presented in Figure 1. Graphing the trabecular density (the "x" coordinate) against the 128 consecutive computer-selected points (the "y" coordinate)

Table 1
Prevalence of Bone Structure Alteration, Osteosclerosis and Fluorosis in the Investigated Group

<table>
<thead>
<tr>
<th>(N = 297)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabeculae Pattern</td>
<td>Thickened Condensed 12.5</td>
</tr>
<tr>
<td></td>
<td>Thickened Dispersed 5.4</td>
</tr>
<tr>
<td></td>
<td>Fine Condensed 2.1</td>
</tr>
<tr>
<td></td>
<td>Fine Dispersed 6.0</td>
</tr>
<tr>
<td>Osteosclerosis</td>
<td>Grade I 13.9</td>
</tr>
<tr>
<td></td>
<td>Grade II 1.8</td>
</tr>
<tr>
<td></td>
<td>Grade III 0.4</td>
</tr>
<tr>
<td>Fluorosis</td>
<td>Stage 0 44.6</td>
</tr>
<tr>
<td></td>
<td>Stage I 0 20.5</td>
</tr>
<tr>
<td></td>
<td>Stage I 3.6</td>
</tr>
</tbody>
</table>
Figure 1
Radiographs of Aluminum Workers Representing Bone Structure Alteration. From the left: (2226) trabeculae fine and condensed (1484) trabeculae thickened and condensed. Microdensitometric analyses are presented on the graphs and listed below.

The Results of the Microdensitometric Analyses of the Radiographs

<table>
<thead>
<tr>
<th>Number</th>
<th>Width (mm)</th>
<th>Condensation (%)</th>
<th>Height (%)</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2226</td>
<td>17</td>
<td>0.45</td>
<td>51.5</td>
<td>9.89</td>
</tr>
<tr>
<td>1484</td>
<td>11</td>
<td>0.58</td>
<td>49.5</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Fluoride
Table 2

Results of the Microdensitometric Analyses in the Whole Group Investigated (T), and in the Group with Fluorosis (Stage: 0, OI, I) and Control Group (C)

<table>
<thead>
<tr>
<th>Groups</th>
<th>T</th>
<th>F-O</th>
<th>F-OI</th>
<th>F-I</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cases</td>
<td>297</td>
<td>124</td>
<td>56</td>
<td>10</td>
<td>47</td>
</tr>
<tr>
<td>Number of Trabeculae (SD)</td>
<td>11.5 (2.85)</td>
<td>11.3 (2.17)</td>
<td>11.3 (1.32)</td>
<td>9.8 (3.15)</td>
<td>12 (2.23)</td>
</tr>
<tr>
<td>Width (mm) (SD)</td>
<td>0.52 (0.09)</td>
<td>0.52 (0.08)</td>
<td>0.54 (0.1)</td>
<td>0.50 (0.15)</td>
<td>0.50 (0.06)</td>
</tr>
<tr>
<td>Condensation (%) (SD)</td>
<td>47.6 (3.53)</td>
<td>47.1 (9.1)</td>
<td>47.5 (18.8)</td>
<td>44.3 (8.9)</td>
<td>48.0 (8.49)</td>
</tr>
<tr>
<td>Height (%) (SD)</td>
<td>14.7 (4.37)</td>
<td>14.8 (4.7)</td>
<td>14.7 (4.7)</td>
<td>13.5 (5.3)</td>
<td>14.0 (5.05)</td>
</tr>
<tr>
<td>Area (%) (SD)</td>
<td>4.3 (2.13)</td>
<td>4.21 (1.88)</td>
<td>4.6 (2.17)</td>
<td>3.89 (3.86)</td>
<td>4.18 (1.6)</td>
</tr>
</tbody>
</table>

Figure 2

Trabecular Width in the Control Group (C), Aluminum Workers (A), and Groups with Diagnosed Fluorosis: F-O, F-OI, F-I.

Table 2 shows the microdensitometric curve. The computerized analysis is plotted below the densitometric curve. The rectangle indicates the starting and terminating points of the trabeculae. Results of the microdensitometric analysis are presented in Table 2. The study of control groups including unexposed subjects is presented in detail elsewhere (8). It is apparent that, as fluorosis advances, the number of trabeculae decreases and their width increases (stage I, \( p < 0.1 \)), whereas no differences were observed in their height and area (Table 2, Figure 2).

The relation of bone structure parameters to the duration of exposure, the worker's work post in the refinery, and the worker's age were also analyzed. Trabeculae were wider and higher in those subjects with longer exposure to fluoride. No correlation was found relative to the subject's work post. Aging is manifest in decreased density and increased width of trabeculae.

Discussion

Analysis of bone structure is essential to the diagnosis of metabolic bone disease (9). However, precise definition of normal bone structure is almost
impossible from routine radiographs. Especially challenging is the diagnosis of the early stages of industrial fluorosis (3).

Digitalization of the radiographic picture followed by computerized analysis makes precise quantitative assessment possible. Microdensitometric analyses have been applied in osteoporosis and in post-radiation bone changes; however, the computer programs used were entirely different from those in the present study (10,11). The unique algorithm successfully used in this study was based on the physiology of sight and theoretical definition on bone trabeculae. The programs were effective in every radiograph and resulted in the quantitative description of bone structure. This method has been experimentally verified and proved to give reproducible results with acceptable limits of error (6). That test and clinical experience indicate that the most useful criteria in bone structure analysis were the number of trabeculae, their width, and their density.

Conclusion

Microdensitometric measurements make possible the digitalization and numerical analysis of radiographs. The description of the bone pattern can provide precise definition of trabecular features such as width, height, area, and density. Decreased number and increased width of trabeculae were the most characteristic features of fluorosis.

References


**********
SHORT-TERM TOXICITY OF FLUORIDE ION (F⁻) IN SOFT WATER 
TO RAINBOW TROUT (Salmo gairdneri) 
AND BROWN TROUT (Salmo trutta fario) 

by 
J.A. Camargo* and J.V. Tarazona 
Madrid, Spain 

SUMMARY: Short-term static bioassays were conducted to determine the toxicity of fluoride ions (F⁻) in soft water (hardness average value of 22 ppm CaCO₃) to Salmo gairdneri Richardson and Salmo trutta fario Linnaeus. Fry of each trout species were exposed to five different concentrations of sodium fluoride (NaF) and a high concentration of sodium chloride (800 ppm NaCl for S. gairdneri and 1000 ppm NaCl for S. trutta fario) for 8 days. No significant effect on the fish was observed with NaCl. Toxic effects caused by NaF were fundamentally due to F⁻ ions. The LC₅₀ at 96, 120, 144, 168, and 192 h were 107.5, 94.4, 85.1, 73.4, and 64.1 ppm F⁻ for rainbow trout and 164.5, 135.6, 118.5, 105.1, and 97.5 ppm F⁻ for brown trout, respectively. Fry showed hypoexcitability, darkened backs and a decrease in respiration before their death. S. gairdneri was significantly (p < 0.05) more sensitive to F⁻ than S. trutta fario. A MATC of 27.6 ppm F⁻ has been determined for rainbow trout. 

KEY WORDS: Fluoride ion; Salmo gairdneri; Salmo trutta fario; Short-term toxicity; Soft water. 

Introduction 

The fluoride concentration in sea waters normally ranges from 1.2 to 1.4 ppm (1) and most fresh waters contain less than 0.2 ppm F⁻ (2). However, the fluoride concentration in surface waters is increasing as a result of industrial pollution (3), which may generate an acute or chronic toxicity to biological communities. In this respect, McClurg (4) has indicated that freshwater organisms may be far more sensitive to fluoride pollution than those living in sea waters, because the toxicity of fluoride is decreased by the formation of innocuous complexes with one or more ions of sea water (5). 

Toxic effects of fluoride compounds have been described in aquatic invertebrates as Daphnia magna (2,6), Artemia salina (7), Penaeus indicus (4,8) and Hydropsyche spp. (9). It appears that soft water species are more sensitive to fluoride than those in hard or sea water. In fish, the fluoride toxicity may be influenced not only by such common factors as size (10), species (11,12), and physiological state (13,14), but also by the physicochemical characteristics of the water. Thus, the tolerance of fish to fluoride is increased by low temperatures (15,16) and high levels of calcium hardness (17-19). To this effect, Pimentel and Bulkley (20) found that the 96-hour LC₅₀ values for Salmo gairdneri increased from 51 to 193 ppm F⁻ as water hardness rose.

Short-Term F⁻ Toxicity in Soft Water to Trout

from 17 to 385 ppm CaCO₃. Smith et al. (12) concluded that LC₅₀ values Gasterosteus aculeatus and Pimephales promelas varied with the initial water hardness due to the precipitation of calcium and magnesium salts (CaF₂ and MgF₂).

The principal purpose of this study has been to determine the short-term toxicity of fluoride ions (F⁻) in soft water to fry of Salmo gairdneri and Salmo trutta fario, common species in cold-water streams from the Iberian Peninsula, and to study whether there are significant differences between these two trout species in relation to their respective sensitivity to fluoride ions.

Materials and Methods

Fry of rainbow trout (Salmo gairdneri) and brown trout (Salmo trutta fario) were obtained from a Spanish ICONA trout hatchery and were certified as disease free at our CIT-INIA laboratories. No fish died during the transportation. In the laboratory, fish were randomly distributed into test aquaria. Those fry used in the experiments were about two months old and, after fluoride toxicity bioassays, weighed (dry weight) 118.9 ±14.9 mg for S. gairdneri and 123.6 ±20.9 mg for S. trutta fario.

Laboratory bioassays were conducted in glass aquaria each containing 20 L. of dechlorinated Madrid tap water (Figure 1). Necessary water oxygenation and turbulence were produced by an air pump per aquarium. Natural photoperiod was utilized, and water temperature was maintained by means of a cooling unit with thermostat. Test fluoride solutions were prepared from sodium fluoride (NaF pro analysi, Merck), geometrically increasing the concentration with an approximate factor of 1.6.

Hardness, alkalinity, chlorine, chloride, sodium, potassium, amonia, nitrite, pH, water temperature, dissolved oxygen, and conductivity were analyzed at the start and at the end of each toxicity bioassay, using analytical methods described by APHA (21) and Rodier (22). Fluoride concentrations were monitored daily using an Orion model 94-09 specific ion electrode. The trout fry were exposed to sodium fluoride solutions for 8 days.

Methods for these static acute toxicity bioassays were those recommended for standardized laboratory toxicity tests (21,23). A control and five different fluoride concentration aquaria were used per bioassay (Figure 1). Fluoride bioassays were performed in duplicate. Each trout species was separately tested using ten fish per aquarium.

Test organisms were acclimatized to water quality conditions for 4 days prior to fluoride bioassays and were not fed during the acclimatization nor during toxicity bioassays. No fish died during the acclimatization. Dead fish were removed daily during fluoride toxicity bioassays. Sublethal effects were daily checked by comparing fish in fluoride aquaria with those in control aquarium.

The LC₅₀ values at 96, 120, 144, 168 and 192 hour, their 95% confidence limits and χ² values were determined by the method of Litchfield and Wilcoxon (24), using mortalities and mean assay F⁻ concentrations obtained in duplicate for each trout species. Death of fish was defined as the fish was floating upside down and not operculating.
Figure 1
Diagram of Experimental Aquaria System Used for Fluoride Toxicity Bioassay.
\( \therefore \) = control aquarium; 1, 2, 3, 4, and 5 = fluoride aquaria.

The formula of factors (21,24) was applied for obtaining significant (p < 0.05) differences between two test trout species. The Maximum Acceptable Toxic Concentration (MATC) was interpolated as the geometric mean of the lowest concentration having a toxic effect and the highest concentration having no toxic effect (25) after 8 days exposure to fluoride solutions.

To find out whether the toxicity of sodium fluoride was due to fluoride ions, sodium and conductivity toxicity tests were conducted parallel to fluoride toxicity bioassays using sodium chloride (NaCl pro analysi, Merck). For this purpose, 10 fry of each test trout species were exposed to a high sodium chloride concentration for 196 hours: 800 ppm NaCl for rainbow trout and
1000 ppm NaCl for brown trout. These tests were performed in duplicate. Physicochemical parameters were analyzed at the start and at the end of each toxicity test using the same analytical techniques. Possible mortality and sublethal effects were checked every day.

Results

Mean values of water parameters estimated during sodium and conductivity toxicity tests are presented in Table 1. No fish died after 196 hours of exposure to NaCl. Fry showed symptoms of hyperexcitability and hyperventilation at first, returning to their normal state after about 10 hours. However, sublethal effects such as hypoexcitability, darkened backs and a decrease in respiration were not observed during these tests. Values of water parameters did not differ appreciably from those reported for the fluoride toxicity bioassay.

Mean values of water quality parameters analyzed during fluoride toxicity bioassay are presented in Table 2. All values are within water quality criteria for aquatic organisms (26). Ammonia and nitrite were not detected at the start, and no precipitation of fluoride salts was observed during these bioassays.

Mean concentrations of fluoride and sodium, conductivity, and the mortality

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salmo gairdneri</th>
<th>Salmo trutta fario</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Temperature (°C)</td>
<td>15.6 ±0.07</td>
<td>15.9 ±0.14</td>
</tr>
<tr>
<td>Alkalinity (ppm CaCO₃)</td>
<td>37.1 ±1.91</td>
<td>29.2 ±1.27</td>
</tr>
<tr>
<td>Hardness (ppm CaCO₃)</td>
<td>22.3 ±1.16</td>
<td>21.7 ±1.72</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>10.4 ±0.14</td>
<td>10.4 ±0.07</td>
</tr>
<tr>
<td>Chlorine (ppm Cl₂)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Ammonia (ppm N)</td>
<td>0.13 ±0.170</td>
<td>0.08 ±0.110</td>
</tr>
<tr>
<td>Nitrite (ppm N)</td>
<td>0.01 ±0.010</td>
<td>0.01 ±0.006</td>
</tr>
<tr>
<td>Fluoride (ppm F⁻)</td>
<td>0.09 ±0.005</td>
<td>0.08 ±0.011</td>
</tr>
<tr>
<td>Potassium (ppm K⁺)</td>
<td>0.11 ±0.060</td>
<td>0.12 ±0.010</td>
</tr>
<tr>
<td>pH</td>
<td>7.58 ±0.060</td>
<td>7.64 ±0.040</td>
</tr>
<tr>
<td>Chloride (ppm)</td>
<td>511.4 ±26.9</td>
<td>608.7 ±27.3</td>
</tr>
<tr>
<td>Sodium (ppm Na⁺)</td>
<td>239.5 ±21.9</td>
<td>379.0 ±15.6</td>
</tr>
<tr>
<td>Conductivity (µmhos/cm)</td>
<td>705.0 ±21.2</td>
<td>1175.0 ±35.4</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. = Not Detected.
Table 2
Mean Values of Water Quality Parameters (Means ±S.D., n = 24) Analyzed During NaF Toxicity Bioassays.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salmo gairdneri</th>
<th>Salmo trutta fario</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Temperature (°C)</td>
<td>15.3 ±0.22</td>
<td>16.1 ±0.13</td>
</tr>
<tr>
<td>Alkalinity (ppm CaCO₃)</td>
<td>37.5 ±2.09</td>
<td>32.2 ±1.92</td>
</tr>
<tr>
<td>Hardness (ppm CaCO₃)</td>
<td>22.4 ±1.79</td>
<td>21.2 ±2.55</td>
</tr>
<tr>
<td>Chlorine (ppm Cl₂)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Chloride (ppm)</td>
<td>10.0 ±1.22</td>
<td>10.8 ±0.43</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>10.1 ±0.28</td>
<td>10.1 ±0.20</td>
</tr>
<tr>
<td>Ammonia (ppm N)</td>
<td>0.15 ±0.157</td>
<td>0.12 ±0.121</td>
</tr>
<tr>
<td>Nitrite (ppm N)</td>
<td>0.01 ±0.009</td>
<td>0.01 ±0.008</td>
</tr>
<tr>
<td>Potassium (ppm K⁺)</td>
<td>0.08 ±0.020</td>
<td>0.14 ±0.016</td>
</tr>
<tr>
<td>pH</td>
<td>7.58 ±0.179</td>
<td>7.63 ±0.185</td>
</tr>
</tbody>
</table>

N.D. = not detected

obtained during fluoride short-term toxicity bioassays are presented in Table 3. Standard deviations were lower than 10% of their respective mean values. There was no mortality in control aquaria. For the experimental group, the mortality increased with increase in fluoride concentrations. Fry in the fluoride aquaria showed hyperexcitability and hyperventilation at first and, at alternate times during test, hypoxicity, darkened backs and a decrease in respiration before their death. However, these sublethal symptoms were not observed in fry exposed to a mean concentration of 22.3 ppm F⁻.

The LC₅₀ values at 96, 120, 144, 168 and 192 hours, their 95% confidence limits, and χ² values obtained for each test trout species are presented in Table 4. All χ² values were lower than those for p = 0.005, indicating that the data are not significantly heterogeneous. The Maximum Acceptable Toxic Concentration (MATC) after 8 days’ exposure to fluoride solutions was 27.6 ppm F⁻ for rainbow trout.

The significant differences between LC₅₀ values are shown in Table 4. LC₅₀ values for brown trout were significantly (p < 0.05) higher than those for rainbow trout to sodium fluoride for 96, 120, 144 and 192 hours. This indicates that Salmo gairdneri is a more sensitive species to F⁻ ions than Salmo trutta fario.

Discussion

Although it has already been indicated that among the metallic ions, Na⁺ ion has the lowest toxicity for aquatic organisms (27), this study has demonstrated the toxic effect of sodium fluoride on trout species is fundamentally due to fluoride ions.
On the other hand, rainbow trout and brown trout appear significantly more resistant to fluoride ion than freshwater benthic microinvertebrates, since Camargo and Tarazona (9) have estimated the 96 hour LC50 values for F⁻ in soft water to be 26.3, 26.5, 38.5, 48.2 and 44.9 ppm for *Hydropsyche bulbifera*, *E. exocellata*, *H. pellucidula*, *H. lobata*, and *Chimarra marginata* larvae, respectively.

Because a range of widely divergent LC50 values has been reported for fluoride in fish and a direct comparison of LC50 values obtained during different studies is not fit because of the several methods used to report toxic

<table>
<thead>
<tr>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Values of Conductivity, Sodium and Fluoride Obtained in Duplicate During NaF Toxicity Bioassays for Rainbow Trout and Brown Trout.</td>
</tr>
<tr>
<td>c = control aquarium; 1, 2, 3, 4 and 5 = fluoride aquaria.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salmo gairdneri</th>
<th>c-c</th>
<th>1-1</th>
<th>2-2</th>
<th>3-3</th>
<th>4-4</th>
<th>5-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (µmhos/cm)</td>
<td>32.5-30.0</td>
<td>142-145</td>
<td>202-202</td>
<td>325-335</td>
<td>485-495</td>
<td>635-640</td>
</tr>
<tr>
<td>Sodium (ppm Na⁺)</td>
<td>4.8-5.1</td>
<td>26.1-26.4</td>
<td>46.3-44.1</td>
<td>69.7-71.1</td>
<td>114-113</td>
<td>190-190</td>
</tr>
<tr>
<td>Fluoride (ppm F⁻)</td>
<td>0.08-0.09</td>
<td>22.3-22.3</td>
<td>34.4-34.2</td>
<td>57.6-57.3</td>
<td>91.4-91.0</td>
<td>144-146</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-10</td>
<td>20-30</td>
<td>50-30</td>
<td>60-70</td>
</tr>
<tr>
<td>120 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-10</td>
<td>20-30</td>
<td>50-40</td>
<td>70-80</td>
</tr>
<tr>
<td>144 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-10</td>
<td>20-30</td>
<td>60-40</td>
<td>70-90</td>
</tr>
<tr>
<td>168 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>20-10</td>
<td>20-30</td>
<td>60-60</td>
<td>90-90</td>
</tr>
<tr>
<td>192 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>20-10</td>
<td>20-40</td>
<td>70-70</td>
<td>90-100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salmo trutta fario</th>
<th>c-c</th>
<th>1-1</th>
<th>2-2</th>
<th>3-3</th>
<th>4-4</th>
<th>5-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (µmhos/cm)</td>
<td>42.5-40.0</td>
<td>218-213</td>
<td>318-315</td>
<td>485-495</td>
<td>650-665</td>
<td>1075-1025</td>
</tr>
<tr>
<td>Sodium (ppm Na⁺)</td>
<td>7.3-8.3</td>
<td>48.2-48.8</td>
<td>68.2-69.3</td>
<td>111-113</td>
<td>189-190</td>
<td>293-291</td>
</tr>
<tr>
<td>Fluoride (ppm F⁻)</td>
<td>0.08-0.08</td>
<td>33.9-35.0</td>
<td>55.4-53.8</td>
<td>90.3-90.6</td>
<td>146-150</td>
<td>236-228</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-0</td>
<td>20-20</td>
<td>40-30</td>
<td>70-80</td>
</tr>
<tr>
<td>120 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-0</td>
<td>20-30</td>
<td>50-30</td>
<td>80-90</td>
</tr>
<tr>
<td>144 hrs</td>
<td>0-0</td>
<td>0-0</td>
<td>10-10</td>
<td>30-30</td>
<td>60-40</td>
<td>80-90</td>
</tr>
<tr>
<td>168 hrs</td>
<td>0-0</td>
<td>0-10</td>
<td>10-10</td>
<td>30-40</td>
<td>60-50</td>
<td>100-30</td>
</tr>
<tr>
<td>192 hrs</td>
<td>0-0</td>
<td>10-10</td>
<td>20-10</td>
<td>40-40</td>
<td>60-50</td>
<td>100-100</td>
</tr>
</tbody>
</table>

Fluoride
Table 4
LCS₉₀ values, their 95% confidence limits and χ² values obtained for each test trout species.

<table>
<thead>
<tr>
<th>Test Duration</th>
<th>Species</th>
<th>LCS₉₀ (ppm F⁻)</th>
<th>95% C.I. (ppm F⁻)</th>
<th>χ² Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 hours</td>
<td>X</td>
<td>107.5</td>
<td>138.0-83.7</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>124.5*</td>
<td>205.1-131.9</td>
<td>3.41</td>
</tr>
<tr>
<td>120 hours</td>
<td>X</td>
<td>92.4</td>
<td>116.0-73.6</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>135.8*</td>
<td>161.2-114.0</td>
<td>5.40</td>
</tr>
<tr>
<td>144 hours</td>
<td>X</td>
<td>85.1</td>
<td>105.5-68.1</td>
<td>2.88</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>118.5*</td>
<td>149.2-94.1</td>
<td>4.79</td>
</tr>
<tr>
<td>168 hours</td>
<td>X</td>
<td>73.4</td>
<td>96.4-55.9</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>105.1*</td>
<td>134.9-81.8</td>
<td>6.63</td>
</tr>
<tr>
<td>192 hours</td>
<td>X</td>
<td>64.1</td>
<td>82.2-50.0</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>97.5*</td>
<td>123.8-76.8</td>
<td>6.90</td>
</tr>
</tbody>
</table>

* p < 0.05.

Effects, maximum safe criteria of fluoride ion for fish in natural ecosystems have not yet been achieved (26). However, it is evident that rainbow trout and other species of freshwater fish may bear higher fluoride concentrations in hard water than in soft water (12,20). In this sense, it has been reported (16) that fluoride ion may form stable complexes with calcium in blood and bone, and Pimental and Bulkley (20) have suggested that a reservoir of calcium in the water surrounding fish tends to compensate for this loss of calcium and thereby delays toxic effects of fluoride on the organism.

Further research on the toxicity of fluoride ion to freshwater fish should, therefore, be conducted under conditions of highest toxicity, namely, soft water, for obtaining a suitable safe level of F⁻. The estimated 8-day MATC might furnish a preliminary safe criterion for trout species. Nevertheless, chronic toxicity bioassay is needed to improve fluoride quality criteria. To this end, the data obtained from the present work may provide the background for future long-term toxicity research to establish safe fluoride standards for freshwater fish.

References


**********
FLUCTUATION OF FLUORIDE CONCENTRATIONS IN DRINKING WATERS: A COLLABORATIVE STUDY

by

M.J. Larsen, O. Fyerskov, O. Bojen, F. Sunderovitz
D. Lambrau, F. Manji and M. Hobdell
Aarhus, Denmark; Godthab, Greenland; Athens, Greece
Nairobi, Kenya and Dublin, Ireland


This study describes the variations in concentrations of fluoride in drinking water sources in Greenland, Kenya, Greece, Denmark and Ireland. Water samples were collected monthly and shipped to laboratories in Aarhus, Denmark, for analysis. In Narssaq, Greenland, the fluoride concentration of a single piped water supply ranged from 0.3 to 2.8 ppm. Variations were related to climate, precipitation and temperature over the year. The fluoride in water from the Athi River, Kenya, ranged from 0.3 to 1.2 ppm; the higher concentrations were associated with dry seasons. The fluoride concentration in piped water from mountain rivers in Mourjes, Greece, ranged during the year between 1.3 and 2.0 ppm; the changes were apparently unrelated to rainfall. Variations in fluoride concentrations from 0.5 to 3.5 ppm were observed in water from artesian wells in Assiros, Greece. Fluoride in drinking water from boreholes in Boennerup Stand, Denmark, ranged from 1.4 to 2.4 ppm; the variations were unrelated to climate or precipitation. Little variation in fluoride concentrations was found in water from boreholes in either Roedvig or Egens, Denmark. However, water obtained from two artificially fluoridated sources in Ireland showed considerable variations with time, although when samples were pooled relatively constant levels over the year were obtained.

According to the study the results of single fluoride ion measurements from any given source should not be considered a reliable indicator of fluoride exposure to drinking water.

KEY WORDS: Fluoride analysis; Fluoride concentration; Fluoride variation; Water-borne fluoride.

REPRINTS: Dr. M. Joost Larsen, Institute of Oral Anatomy, Dental Pathology and Operative Dentistry, Royal Dental College, Vennelyst Blvd., DK-8000 Aarhus C, Denmark.
INSTRUCTIONS TO AUTHORS

Fluoride, the official journal of the International Society for Fluoride Research (ISFR) publishes quarterly (Winter, Spring, Summer, Autumn) reports on the biological, chemical, ecological, industrial, toxicological and clinical aspects of inorganic and organic fluoride compounds. Papers presented at the annual ISFR conference appear in Fluoride. Submission of a paper implies that it presents original investigations and relevant bio-medical observations. Review papers are also accepted.

PREPARATION OF PAPERS

1. General – No precise limit is given on the length of the paper. However, it should be written concisely in English, submitted with a copy, doublespaced with generous margins. Measures are given in metric system (SI).

2. Title – A concise but informative title must be followed by the name of author(s), the location and state (country) where the research was carried out. The name and address of the institution where the work was done should appear at the bottom of the first page.

3. Summary – All papers should begin with a brief, factual summary.

4. Key Words – include major themes or research subjects.

5. Introduction – Following the summary, a short introduction should state the reason for the work with a brief review of previous works on the subject. References are given by numbers in parentheses.

6. Materials and Methods – should be condensed; however, if the methodology is new or developed by the author(s) it can be more detailed.

7. Results – should contain the direct conclusions of the experimental work.

8. Discussion – deals with the general conclusions referring to other work on the subject, and whether the experimental results agree or disagree with previous work. In short papers, Results and Discussion may be combined.

9. Abbreviations or Acronyms – must be defined either parenthetically or in a footnote when they first appear.

10. Bibliography – should be arranged according to the order in which the articles are cited in the text (not alphabetically). An example follows:


For books, the title, editor, publisher, location and year of publication, and pages should be given.