Institute for
Environmental Research

Ministry of Health and
Medical Education

Tehran University of
Medical Sciences

INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

XXXIst CONFERENCE
ADVANCES IN FLUORIDE RESEARCH

PROGRAM AND ABSTRACT BOOK

TEHRAN, IRAN

2013

Professor Henri Moissan
Born: September 28, 1852, Paris, France
Died: February 20, 1907, Paris, France

Winner of the Nobel Prize in for Chemistry 1906
In recognition of the great services rendered by him in his investigation and
“isolation of the **element fluoride”**

Organizers

Institute for Environmental Research (IER), Tehran University of Medical Sciences
International Society for Fluoride Research
In cooperation with
Iranian Association of Environmental Health (IAEH)
Bushehr University of Medical Sciences
Environmental Health Research Network (EHRN)

CONFERENCE ORGANIZING COMMITTEE

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Department of Environmental Health Engineering, School of Public Health and Institute for Environmental Research. Tehran University of medical sciences (Tums), Tehran, Iran

Scientific Secretary

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Executive secretaries

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Center for air pollution research, Institute for Environmental Research (IER), Tehran University of Medical Science, Tehran, Iran
Department of Environmental Health Engineering, Shiraz University of Medical Science, Shiraz, Iran
Fatemeh Momeniha (MSc)
Center for solid waste research, Institute for Environmental Research (IER), Tehran University of Medical Sciences

Keynote Speakers

Prof. Jörg Spitz (Germany)

Dr. Masashi Tsunoda (Japan)

Dr. Bruce Spittle (New Zealand)

Prof. Jungdong Wang (China)

Prof. Susheela Andezhath Kumaran (India)

Prof. Anna Strunecka (Czech Republic)

Prof. Elian Dahi (Tanzania)

Dr. Shanti Lal Choubisa (India)

Dr. Declan Waugh (Ireland)

Dr. Ümit Demirel (Turkey)

Prof. Zhi-Zhong Guang (China)

WELCOME

Dear Participants and Guests,

On behalf of the Conference Organizing Committee, I would like to welcome you all to Tehran and the Islamic Republic of Iran for our XXXIst Conference of the International Society for Fluoride Research. You are all most welcome and we are very pleased that you have been able to come to share in presenting and discussing your research on fluoride.

This is the first conference of the Society to be held in the Islamic Republic of Iran and having it here is a recognition of the importance of the fluoride research being done in our country and the growing recognition of the effects fluoride may have on the health of humans, animals, and plants. Studies from this region have identified areas with naturally high levels of fluoride with detrimental effects on health including dental and skeletal fluorosis and impairments of intelligence. At the same time, there is discussion as to whether the intake of fluoride or the level of fluoride in drinking water in some areas is too low for optimal health. The quarterly journal of our Society, Fluoride, is currently in its 46th year of publication and contains discussion on whether or not fluoride is an essential nutrient or trace element for which a small amount is necessary for proper growth, development, and reproductive functioning. At present the research indicates the fluoride is not an essential nutrient and that there is no human need for any fluoride for proper growth and development. However, the question has arisen as to whether or not increasing the level of fluoride in the drinking water in some parts of the country would be beneficial for dental health. As a Society, we do not have a view on water fluoridation which remains controversial, with respect to its ethics, safety, and efficacy. This is reflected in various countries having completely different policies towards fluoride in water. In many countries, including the People's Republic of China and India, the focus is on understanding the mechanisms of fluoride toxicity and finding ways to reduce the burden of fluoride on human, animal, and plant life. Finding safe sources of water and reducing the level of fluoride in drinking water is an important issue in many countries including Iran, India, and Tanzania. Where environmental pollution, such as from coal burning, is a problem, as in some areas of China, finding nutritional measures to reduce the level of toxicity has economic importance. In contrast, in some other countries, such as in New Zealand, Australia, Canada, and the United States of America, the health authorities see the fluoride level in the drinking water, in some areas, as being too low and needing to be increased to improve dental health. Similar considerations have been aired in Iran.

In our conference we will have an opportunity to study the scientific evidence on the biological effects of fluoride together with the various measures being used to remove fluoride from drinking water with examples from both Tanzania and Iran. As well as studies of the effect of fluoride on IQ, there is growing concern about a rise in the incidence of various neurodevelopmental disorders including the autism spectrum disorders. The effects of fluoride on fertility, renal function, and cardiac function have also emerged as being of fundamental importance. Inflammatory respiratory diseases, including asthma and chronic obstructive pulmonary disease, are important causes of morbidity world-wide and we will have an opportunity to discuss the role fluoride may have in these conditions. The interaction of fluoride with other elements including metals, the formation of aluminofluoride complexes, and how these may affect G-proteins will be other important areas for consideration.

We welcome all of the platform and poster presentations that will be made at the conference. They will all contribute to our growing understanding of the effects that fluoride may have. We are grateful for our keynote speakers coming to Tehran to share their knowledge and research.

May I also acknowledge the hard work that has been done by the conference organizing committee? I am grateful for the leadership of our conference chairman, Professor Alireza Mesdaghinia. Our academic committee has been energetic in helping produce a substantial scientific programme with approximately 62 presentations in addition to an anticipated 11 keynote addresses. Our thanks go to: Professor Simin Nasseri, Professor Kazem Naddafi, Professor Masud Younesian, Dr. Ramin Nabizadeh, Dr. Kamiar Yaghmaeian, Dr. Noushin Rastkari, Dr. Gholamreza Jahed, Dr. Mahmood Alimohammadi, Dr .Nabi Shariatifar, and Dr. Mohammad Hadi Dehghani. Our executive secretaries Dr. Abbas Shahsavani and Eng. Fatemeh Momeniha have worked tirelessly with the myriad of tasks involved in organizing the conference and I thank them sincerely. We are again grateful to Professor Jörg Spitz for his sponsorship of the Niloufer Chinoy awards for the best oral and poster presentations at the conference by researchers aged less than 35. We are also appreciative of the advice of Professor Emeritus Albert Burgstahler and Dr Bruce Spittle. We regret that Professor Emeritus Burgstahler is not able to be with us and our thoughts have been with him during his time of grave illness.

We appreciate that you have come here, not only from different regions of Iran, but from many parts of the world including from the People's Republic of China, the Czech Republic, Germany, India, the Irish Republic, Japan, New Zealand, Pakistan, Tanzania, Thailand, England and Turkey.

I welcome you again to the Islamic Republic of Iran, I trust that our conferring together will be beneficial, and that, at the end, you will take home with you warm memories of our time together as well as an appreciation of this seat of civilization.

Dr. Amir Hossein Mahvi
Scientific Secretary, XXXIst ISFR Conference

HISTORICAL PERSPECTIVE OF ISFR

Event	Venue	Date
Founding of the society	Bern, Switzerland	1962
1st Conference	Frankfurt am Main, Germany	1967
2nd Conference	Barcelona, Spain	1969
3rd Conference	Vienna, Austria	1970
4th Conference	Hague, Netherlands	1971
5th Conference	Oxford, England	1973
6th Conference	Williamsburg, VA, USA	1974
7th Conference	Zandvoort, Netherlands	1976
8th Conference	Oxford, England	1977
9th Conference	Freiburg, Switzerland	1978
10th Conference	Oxford, England	1979
11th Conference	Dresden, England	1981
12th Conference	St. Petersburg, FL, USA	1982
13th Conference	New Delhi, India	1983
14th Conference	Morioka, Japan	1985
15th Conference	Logan, UT,USA	1986
16th Conference	Nyon, Switzerland	1987
17th Conference	Budapest, Hungary	1989
18th Conference	Arcata, CA,USA	1990
19th Conference	Kyoto, Japan	1992
20th Conference	Beiging, China	1994
21th Conference	Budapest, Hungary	1996
22th Conference	Bellingham, WA, USA	1998
23th Conference	Szczecin, Poland	2000
24th Conference	Otsu, Japan	2001
25th Conference	Dunedin, New Zealand	2003
26th Conference	Wiesbaden, Germany	2005
27th Conference	Beiging, China	2007
28th Conference	Toronto, Canada	2008
29th Conference	Jaipur, India	2010
30th Conference	Szczecin, Poland	2012
31th Conference	**Tehran, Iran**	**2013**

General information

Conference Date
October 18–21, 2013

Conference Venue
9th Floor, Tehran University of Medical Sciences (TUMS),Ghods St., Keshavarz Blvd.,Tehran, Iran.

Registration and Information
The welcome reception and the opening ceremony will commence at 16:00 – 18:00 on October 18. The registration desk is located on ninth floor of the Tehran University of Medical Sciences. The conference will finish at18:00 on October 21.

Conference registration fees
$200 (standard registration)
$100 (student registration)
In order to register as students' registration condition, the attendee must provide the conference office with the proof of identity i.e. university ID card or similar.

Registration fee structure
Full conference registration includes: name badge, conference materials, and all conference sessions, opening plenary, receptions during the conference, 3 luncheons, City Tour, 2 dinner and coffee breaks.

Niloufer Chinoy Awards
Two Niloufer Chinoy Awards, each of € 1000, as announced by Professor Jörg Spitz in October 2007 to honor late Professor Niloufer Chinoy, will be given for the best oral and poster presentation at the conference by researchers, aged less than 35 at the closing date for the abstracts, Aug. 30,2013.
The judging of the platform and the poster presentations will be done by two teams of three judges. Scoring will be based on 50-point scale with a maximum of 10 points for quality in each of the following five Categories: 1.content 2. Presentation 3. Importance 4. Relevance to society and 5. Defense in discussion.
Entitled researchers wishing to participate in the Niloufer Chinoy Awards competition are requested to inform the Organizers of this intention before 17:00 on Friday, October 18th at the registration desk.

Conference Secretariat & Contact

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XXXI[st] CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH
OCTOBER 18-21, 2013
TEHRAN- IRAN

Friday, October 18th, 2013				
16:00- 17:00		Registration/ Information		
17:00-17:15		Opening Ceremony		
17:15-17:30		Inaugural Presentation: Dr. Ali. Jafarian, Chancellor Tehran University of Medical Sciences		
17:30-17:45		Inaugural Presentation: Dr. Mesdaghinia, Chairman Conference		
17:45-18:00		Inaugural Presentation Dr. Mahvi, Scientific Secretary Conference		
Platform Session 1:Fluoride and the gastrointestinal and endocrine systems				
Articles code	Time	Title	Presenter	Country
keynote speaker	18:00-19:00	The nature deficit disorder - why fluoride toxicity becomes even more critical in the 21st century	Prof. Jörg Spitz	Germany
A-10-100-1	19:00-19:15	Fluoride reduces vitamin D and glucose tolerance in school children	Dr. Anurag Tomar	India
	19:15- 20:00	**Coffee break**		
Saturday, October 19th, 2013 **Platform Session 2: Fluoride and the renal, respiratory, and cardiovascular systems**				
keynote speaker	8:30- 9:30	Experimental approach to the toxicity of fluoride among a sensitive population: use of rodents with renal function impairment	Dr. Masashi Tsunoda	Japan
keynote speaker	9:30- 10:30	Epidemiology of fluoride: how fluoride contributes to inflammatory respiratory diseases, including asthma and COPD	Dr. Declan Waugh	Ireland
	10:30- 11:00	**Coffee break**		

A-10-59-1	11:00-11:20	Effect of sodium fluoride on the oxidative stress and expression of bcl-2 and Bax in rat myocardium	Dr. Xiaoyan Yan	China
A-10-63-1	11:20- 11:45	An environmental study: is there correlation between fluoride content of drinking water and hypertension?	Dr. Dobaradaran	Iran
	11:45-12:00	Q&A/Conclusion		
Platform Session 3: Fluoride and the eye				
A-10-102-1	12:00-12:15	Fluoride increases oxidative burden in cataract genesis: an *in vitro* and *in vivo* study	Dr. Swati Tomar	India
A-10-108-1	12:15-12:30	In-vitro study of fluoride induced biochemical changes in goat eye lens	Dr. Sudhanshu Mishra	India
A-10-116-1	12:30-12:45	Fluoride affect quality of vision: an observational study	Dr. Swati Tomar	India
	12:45-13:00	Q&A/Conclusion		
	13:00-14:00	**Lunch**		
Platform Session 4:Fluoride and the reproductive system				
keynote speaker	14:00- 15:00	A retrospective look at three decades of research on fluoride toxicity	Dr. Jungdong Wang	China
A-10-107-1	15:00- 15:15	Fluoride influences metal antagonistic effect in seminal plasma	Dr. Dushyant Singh Chauhan	India
A-10-117-1	15:15- 15:30	Endemic fluoride elevates increased oxidative burden in cord blood	Dr. Dushyant Singh Chauhan	India
	15:30-16:00	**Coffee break**		
Poster: 16:00- 17:00 **Poster Session 1: Fluoride levels in milk, water, and plants**				
A-10-88-2		Determination of fluoride concentration in water and breast milk in Golestan province in Iran	H. Faraji	Iran
A-10-85-1		Evaluation of fluoride content of groundwater resources and the role of geologic formations in Khosuye-hajiabad plain, Zarrindasht, Iran	F. Momenifar	Iran

A-10-88-1		Determination of fluoride concentration in breast milk in Golestan city in Iran	H. Faraji	Iran
A-10-84-1		Investigation of fluoride distribution pattern in Ghir plain aquifer, Fars province, Iran	A. Momtaz Jahromi	Iran
A-10-70-1		Fluoride concentration level of the Persian gulf shore in Bushehr, Iran	M. Khorsand	Iran
A-10-74-1		Evaluation and comparison of fluoride level in bottled, distribution network and decentralized municipal desalination plant water in Bushehr Iran	R. Hayati	Iran
A-10-61-1		Level of fluoride content in medicinal plants commonly consumed in Iran	Dr. Rastkari	Iran
A-10-60-1		Assessment of fluoride level in drinking water resources of Babol rural regions	H. Faraji	Iran
A-10-124-3		Study of co- existence of fluoride and other mineral contaminants in drinking water (a review study)	S. Rahimi	Iran
A-10-132-1		Fluoride in drinking water of West Azerbaijan Province, Iran	Dr. Mosaferi	Iran
Poster Session 2: Fluoride and teeth				
A-10-97-1		Knowledge, attitude and self-reported practice of fluoride among dentists	Dr. Pakdaman	Iran
A-10-65-1		The study of fluoride concentration in Sepidan drinking water and its relation to DMFT index in 15-18 year-old students	A. Azhdarpoor	Iran
	17:00- 22:00	City Tour/Banquet		
Sunday, October 20th, 2013 **Platform Session 5:Fluoride, bones and teeth**				
keynote speaker	8:30- 9:30	Dental caries in New Zealand in 2011: the effects of socioeconomic status and water fluoridation	Dr. Bruce Spittle	New Zealand
keynote speaker	9:30- 10:30	Chronic fluoride intoxication in diverse species of domestic animals	Dr. Shanti Lal Choubisa	India
	10:30- 11:00	**Coffee break**		
keynote speaker	11:00-12:00	How Fluoride toxicity and associated issues affecting maternal & infant health can be mended	Prof. Susheela Andezhath Kumaran	India

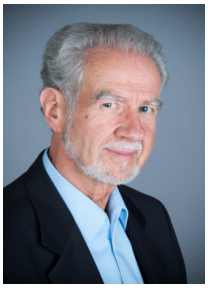
A-10-80-1	12:00-12:15	Association of ERK1 polymorphism and fluorosis in coal-burning of Guizhou province	Prof. Zhi-Zhong Guang	China
A-10-67-1	12:15- 12:30	Effects of laser-assisted fluoride therapy on enamel demineralization	Dr. Maryam Serajzadeh	Iran
A-10-101-1	12:30- 12:45	Biophysical and biochemical markers of skeletal fluorosis	Dr. Mukesh Tiwari	India
	12:45- 13:00	Q&A/Conclusion		
	13:00-14:00	**Lunch**		
Platform session 6 (Part 1): Fluoride and the brain				
keynote speaker	14:00- 15:00	Aluminofluoride complexes—the most dangerous combination of fluoride and aluminium	Prof. Anna Strunecka	Czech Republic
A-10-68-1	15:00- 15:15	Proteomic analysis of hippocampus and cortex in mice exposed to fluoride and lead	Ruiyan Niu	China
A-10-121-1	15:15- 15:30	Influence of age on fluoride induced neurochemical and ultrastructural changes in rat central nervous system	Dr. Sandeep Tripathi	India
A-10-118-1	15:30- 15:45	Fluoride influences aluminum induced behavioral and biochemical changes in rat central nervous system	Dr. Sandeep Tripathi	India
	15:45-16:15	**Coffee break**		
Poster: 16:15- 17:15 **Poster Session 3 (Part 1): Fluoride removal from water**				
A-10-76-1		Fluoride removal from aqueous solutions using shrimp shell as a cost benefit biosorbent	F. Elmi	Iran
A-10-75-1		Fluoride biosorption from aqueous solutions by sargassum hystrix algae	S. Noshadi	Iran
A-10-66-1		Fluoride biosorption from aqueous solutions using moringa oleifera as	M. Kakuee	Iran
A-10-72-1		Fluoride removal from aqueous solutions using Padina sanctae crucis algae as biosorbent	M. Keshtkar	Iran
A-10-38-1		Removal of fluoride from urbane drinking water by bone-char	Dr. Giti Kashi	Iran
A-10-103-1		Performance comparison of electrocoagulation process by using aluminum and iron electrodes on defluoridation of drinking water	Mohammad Mollamahmoudi	Iran
A-10-124-2		A comparative study for excess fluoride removal efficiency by different processes in drinking water supplies (a review study)	S. Rahimi	Iran

A-10-98-1		Modified eggshell powder as an adsorbent for removal of fluoride from aqueous solution	Sara Sadat Hosseini	Iran
A-10-40-1		The survey on the modified sorghum and canola performance as natural biosorbents in biosorption of fluoride from water isothermic and kinetic modeling	Davood Balarak	Iran
A-10-93-1		Investigation of modified montmorillonite by polyethyleneimine to fluoride removal from aquatic medias	Azam Nadali	Iran
	18:00- 21:00	Dinner		
Monday, October 21th, 2013 **Platform session 6 (Part 2): Fluoride and the brain**				
keynote speaker	8:30- 9:30	The potential role of fluoride in autism spectrum disorders	Prof. Anna Strunecka	Czech Republic
keynote speaker	9:30- 10:30	Impaired mitochondrial dynamics in brains, kidney and liver of rats with chronic fluorosis	Prof. Zhi-Zhong Guang	China
	10:30-11:00	**Coffee break**		
A-10-95-1	11:00-11:30	Investigation of intelligence quotient in children age 9-12 years exposed to high and low fluoride in drinking water in west Azerbaijan province, Iran	Dr. Mahvi	Iran
A-10-106-1	11:30-11:45	Correlation of acetylcholinesterase activity and cognitive impairment in fluoride exposed adult population	Dr. Vivek Pratap Singh	India
	11:45-12:00	Q&A/Conclusion		
Platform Session 7:Fluoride and the environment				
keynote speaker	12:00- 12:50	25 Years of Defluoridation Technology Project	Prof. Elian Dahi	Tanzania
A-10-40-2	12:50- 13:00	Kinetics modeling and isotherms for adsorption of fluoride from aqueous solution by modified Azolla filiculoides	Davood Balarak	Iran
	13:00-14:00	**Lunch**		
keynote speaker	14:00- 15:00	Are we aware of the fluorosis problem in our countries, including Turkey?	Dr. Ümit Demirel	Turkey
A-10-104-1	15:00- 15:15	Using a Geographic Information System to Identify areas at risk for Fluorosis in Tambon Mae Pu Kha and Amphoe Sankampaeng, Chiang Mai Province, Thailand	Dr. Wuttichai Choompolkul	Thailand
A-10-30-1	15:15- 15:30	Fluoride excess in ground water of Dungarpur (India): seasonal	Dr. Gyan	India

		fluctuation and clinical manifestations	Vikash Mishra	
	15:30-16:00	**Coffee break**		
Poster: 16:00- 17:00 **Poster Session 3 (Part 2): Fluoride removal from water**				
A-10-63-4		Optimization of fluoride adsorption onto carbonized palm leaves by experimental design	M.Khorsand	Iran
A-10-63-3		Adsorptive removal of fluoride from aqueous solution over modified palm leaves: an optimization study	A Akbarpour	Iran
A-10-63-2		Removal of fluoride from aqueous solution by using response surface methodology	A Akbarpour	Iran
A-10-94-2		Investigation of Fe_3O_4 nanoparticles coated on powder activated carbon efficient in the removal fluoride from aqueous solution	Kani Golzar F Ahmadi	Iran
A-10-94-1		Removal of fluoride from aqueous solution by acid-treated clinoptilolite	Mohammad Molla Mahmudi	Iran
Poster Session 4: Fluoride and various biological factors				
A-10-69-2		RT-PCR amplification of coat protein gene of citrus psorosis virus in Iran	Saeedeh Pourhamedi	Iran
A-10-69-1		Optimization of total RNA extraction method for molecular studies of Citrus psorosis virus in citrus	Saeedeh Pourhamedi	Iran
A-10-81-1		Evaluation of forces applied on selective joints and muscles of drivers during clutching of MF285 and MF399 tractors	H. Falahi	Iran
A-10-124-1		Study of fluoride health effects in drinking water supplies in Sarayan city in Iran	S. Rahimi	Iran
A-10-131-1		Weight and length at birth and their relationship with their mothers fluoride exposure from drinking water in west Azerbaijan province, Iran	S. Karimzadeh	Iran
A-10-95-2		Investigation of hypertension in people exposed to high and low fluoride in drinking water in west Azerbaijan province, Iran	M. Aghaei	Iran
	17:00- 17:30	Judges deliberations		
	17:30- 18:00	Closing ceremony and Niloufer Chinoy awards presentation		

Platform Session 1

Fluoride and the gastrointestinal and endocrine systems



Prof. Dr. Jorg Spitz

THE NATURE DEFICIT SYNDROME - WHY FLUORIDE TOXICITY BECOMES EVEN MORE CRITICAL IN THE 21st CENTURY

According to the results of modern anthropological studies, the origin or evolution of mankind (or humans, *Homo sapiens*) from primitive tool-making hominids on planet Earth extends back several million years. During these long periods of time, humans have been constantly exposed to toxic materials in their environment. However, mankind has not only survived but spread all over the globe. The reason for this unequaled success story lies in the skills of the ingenious human body which is able to compensate for negative impacts as long as sufficient positive (natural) factors are present to maintain health. This principle of nature has worked for eons of time—until the last century!

To understand the dependency of the human body on the resources of its environment we have to go back the long way of evolution to the very beginning of life on our planet (Fig. 1).The single cell swimming in the ancient ocean got everything it needed from the circum-fluent water and excreted everything no longer needed to this environment. This exchange as the basic principle of life has not changed since then, even though multicellular plants and animals as well as the human race have evolved.

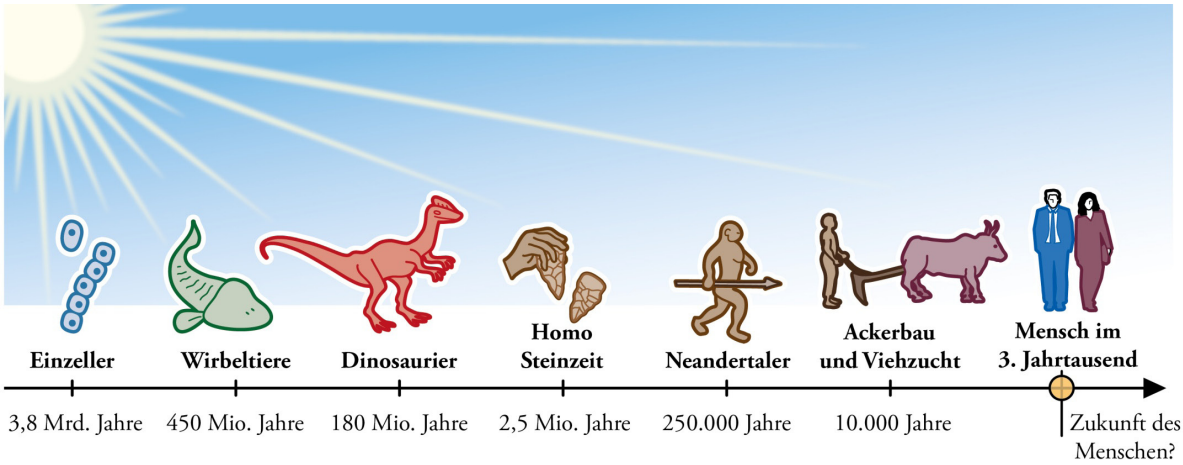


Fig 1: Time course of evolution from single cells to the human beings as symbiotic communities (copyright mip)

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While there is a growing body of evidence that our children need the contact to a natural environment to develop their mental and physical abilities (Louv 2010) little attention is paid to the natural environment as a resource for health in adulthood. A simple example for this thesis is the large surface of our lungs and even more of our gut. Every building block of our body comes from the environment and has to pass through our gut. Without a sufficiently large surface in our lungs to make use of the oxygen in the surrounding air we are going to suffocate. Evolution has shaped the human body according to the natural environment. Each change in the environment automatically influences the efficacy of this relationship. Technical progress has extensively changed this relationship – unfortunately in an unhealthy manner as I will show.

But before we go into these details we have to realize the latest results of scientific research with regard to the construction of our body. We are individual but not single beings. Instead we are composed not only of billions of human cells but also trillions of bacteria and viruses living on our skin and in our gut (Biagi et al. 2012). The DNA of these "guests" in our body weighs more than the human DNA. The genetic research of the fungal species we are hosting on top of the bacteria and viruses has just started. In consequence our body is a huge community of living beings, a microcosm in the macrocosm of our universe.

The reason for the evolution of this symbiotic community is still under research. But obviously bacteria and viruses were dominating the world when the human evolution started. So this evolution could only take place together with supporting friendly bacteria and viruses. Otherwise the organism was doomed to perish. Meanwhile we could learn that the guests in our gut (microbiota) are not idle but influence our immune system (Relman David A. 2011) as as well as our brain, our mood and our wellbeing in every sense (Gonzalez et al. 2011).

All these "guests" in and on our body are also depending from our environment, i.e. with regard to their food (Muegge et al. 2011). If our nutrition does not have the correct composition, the bacterial flora in our gut is likely to starve and die. With this background also the role of (man-made) antibiotics has to be redefined as their application obviously changes the amount and diversity of our gut microbiota resulting in more harm than benefit for us.

Taking all these facts together there is a growing body of alarming evidence that modern chronic diseases like myocardial infarction, stroke, diabetes, osteoporosis, and cancer, plus neurological disorders like attention deficit syndrome, multiple sclerosis, and dementia, are promoted and may be even due to modern technical achievements that have profoundly changed our environment in two ways: First, there is a massive destruction by an unprecedented growth in exposure to a large list of pesticides (Martins et al. 2013; Betsy et al. 2013) and toxic materials like fluoride and aluminum (Strunecka A and Patocka A 2002) .

As this process has taken place in all so-called "civilized nations" the individual person has almost no chance to avoid the contact with these technical achievements: most people eat

highly processed food loaded with the already mentioned pesticides as well as toxic materials and breathe contaminated air. This is the reason why it is so difficult to behave in a healthy way different from the mainstream. Meanwhile we know that the exposure to these technical products provokes not only direct toxicity to cells but in addition induces endocrine disruption by which almost all organs may be affected. Typical examples are disturbed function of the thyroid gland and infertility of the reproductive organs.

Unfortunately there is a second effect of the technical progress on the environment in the civilized nations which so far has not been remedied. The lifestyle changes have led to a serious loss of essential ingredients for good health in large segments of the population: physical exercise, micronutrients, essential fatty acids from plants and animals, and vitamin D—the "sunshine" hormone—as well as the dissipation among many people of close social bonds of performing music and singing, to mention but a few. The deficit of these resources results primarily in a dysfunction of the complicated cell signaling in the body similar to endocrine disruption and in addition leads to inheritable changes of our genes! This phenomenon is part of the new field of scientific studies in "epigenetics" research. Altogether I have found more than 20 resources which are lost due to our modern lifestyle (Table 1). I have called this complex interaction between the shortage of the natural ressources of the environment and our body "Nature –Deficit-Syndrome". Some of these resources are physical phenomena like gravity, the magnetic field of the Earth or the solar radiation. Others have been part of the natural nutrition and the third group concerns mental factors.

Physical Factors	Biological Factors	Mental Factors
gravity	micronutrients	meaningful work
magnetic field	minerals	social bonds
sun light	fat, protein, carbohydrates	spirituality
oxygen	energy	music and singing
clear water	ess. amino acids	meditation
electric potenial of the earth	symbiosis bakteria, viruses, funghi in our gut	senses and perception
silence/darkness	hunger and fasting	rest and recreation

Table 1: List of environmental factors which have become lost for the majority of people in civilized nations, by that leading to the "Nature-Deficit-Syndrome".

In the first approach it may be difficult to understand why these factors should have become lost. A typical example is the loss of gravity effects by our sedentary lifestyle (Balboa-Castillo et al. 2011; Berry et al. 2011). As we still move a little bit now and then indeed the everyday effect is not visible. But this situation changes dramatically if we go to space and leave grav-

ity completely behind us as it happened to the astronauts. They were young well-trained and healthy men when they started for their mission in space and came back with brittle bones and shrunken muscles eight weeks later - unable to stand straight or to walk. Just because they lost gravity - a physical force nobody thinks about in daily life on earth (Vernikos J et al 2010).
The same is true with the solar UVB radiation (Spitz J 2012). This small band of radiation is necessary to induce the production of vitamin D in our skin. This service of the sun has worked for millions of years until we turned our back to the sun and preferred to stay in buildings and cars almost the whole day long. But vitamin D is urgently needed in all cells as in its active form it is a hormone and not a vitamin. Up to thousand genes are switched on and off by means of vitamin D (Hossein-nezhad et al. 2013).
Another example are the micronutrients (Andriantsitohaina et al. 2012; Castaner et al. 2012; Fouad et al. 2013; Pallas et al. 2013) and certain fatty acids (Das 2011; Lawrence 2013) in our nutrition which are as necessary for the cell metabolism as vitamin D. Above all they are found in berries, nuts and vegetables - as long as the food is original (natural) and not processed, because processing food either destroys the micronutrients or removes essential fatty acids because they could become rancid and by that shorten the shelf life of the product.
Finally there are the mental aspects to be discussed. Most of us do no longer produce any music by themselves but listen to recorded music or songs produced by somebody else. Singing has been an especially important source of mental well-being (Gale et al. 2012; Grape et al. 2003). Singing produces hormones in the brain which are needed in the rest of the body (i.e. oxytocin) (Kim et al. 2013; Ebner et al. 2013; Japundzic-Zigon 2013). On the other hand a similar but negative influence on the brain occurs for all the people living as singles (Berry et al. 2012; Pulkki-Råback et al. 2012) or are working in a non-meaningful job (Arnold et al. 2007; Hsee et al. 2013).
An increasing number of epidemiological studies show that many of these resources are missing in the larger part of the population in civilized nations. The developing countries are following them in step. So putting things together it is the exposure to toxic industrial products like fluoride together with the rising loss of resources which corrupt our body and make it decompensate in its functions. The above mentioned chronic diseases are the result of this unhealthy combination.
But there is still another important aspect to mention: most people lose several natural resources and are exposed to several toxic products at the same time. This is most critical because the negative effects of risk factors do not simply add up but multiply. This has been imposingly shown in a large study of 30,000 patients who suffered from myocardial infarction world wide (Yusuf S et al 2004). The researchers found that nine risk factors were responsible for the development of the coronary artery disease - without any genetic influence. Meanwhile these risk factors are well known: physical inactivity, overweight, tobacco abuse, stress etc.

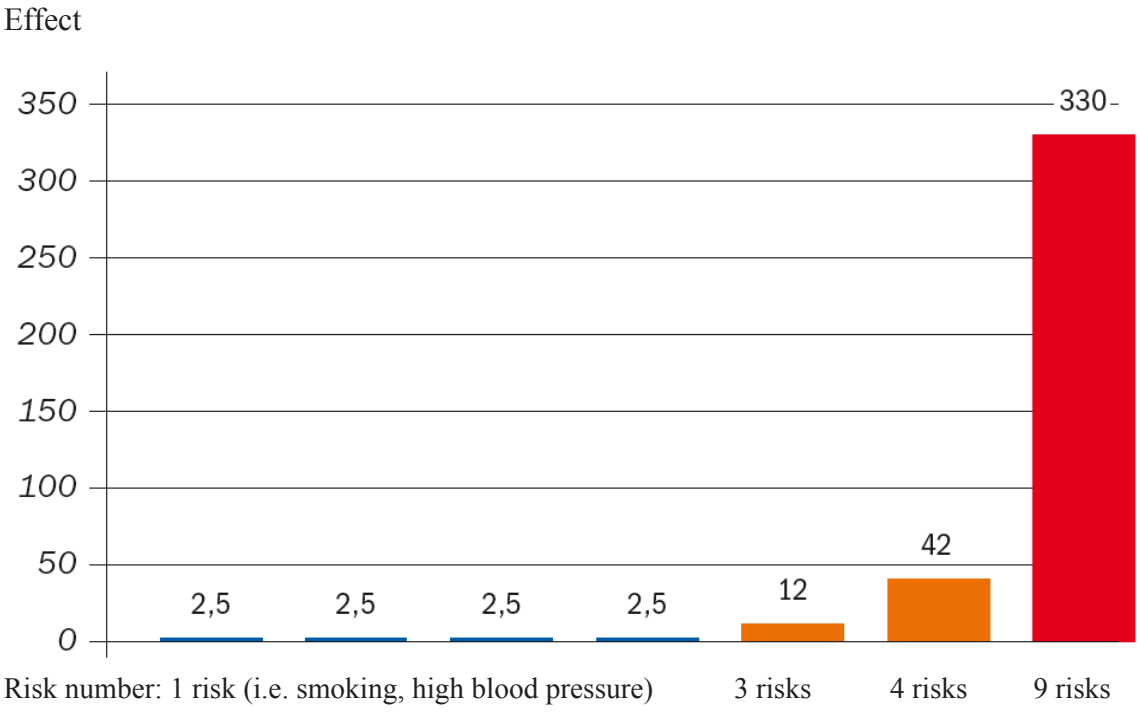

Fig. 2: Steeply rising effects on the probability of developing myocardial infarction, fueled by the number of risk factors (Yusuf S et al 2004)

While in general every factor elevated the risk for myocardial infarction by 2.5 the combined effect of four risks did not result in a tenfold but in a 42 fold increase (Fig. 2). All nine risk factors together elevated the probability for a myocardial infarction by a factor of 330! This example highlights why so many people in the civilized nations develop and die from chronic diseases: their lifestyle induces the loss of several resources and they are exposed to several toxic products at the same time which leads to a high-risk constellation.

Hard to believe, but obviously true: one way to avoid or solve various health problems is a change of lifestyle including a simple nutritional approach! Our beloved late colleague, Professor Emerita Niloufer J Chinoy, Gujarat University, Ahmedabad, India (October 17, 1939 – May 8, 2006), whose family were originally Parsee from Iran, gave an important example: eating the right combination of fresh foods with abundant micronutrients and high antioxidant capacity significantly helps to compensate against many of the adverse effects of endemic fluorosis (Chinoy NJ 2004a; Chinoy NJ 2004b). The same principle largely reduces fluoride toxicity in pregnant women as demonstrated by the results of Prof. A.K. Susheela and her Fluorosis Research and Rural Development Foundation in Delhi (Susheela AK 2010, Susheela AK 2007).

These examples of members of the International Society for Fluoride Research are in no way exceptional cases. In a prospective evaluation of a representative cohort of 23,000 healthy German citizans, aged 45 years at the beginning of the study, the researchers looked for four

aspects of a healthy life style: no tobacco smoking, more than 3.5 hours of physical activity per week, not being overweight and eating healthy food (Mediterranean diet). All together these are just some basic aspects of a healthy life style. But after eight years the results were more than basic: only 9% of the German population were free from risk factors. But those who did had a huge benefit: a reduction of diabetes risk by 93%, a reduction in coronary heart disease risk by 81% and a reduction of the risk of all chronic diseases by78% (Ford et al. 2009). Similar results are reported by American studies.

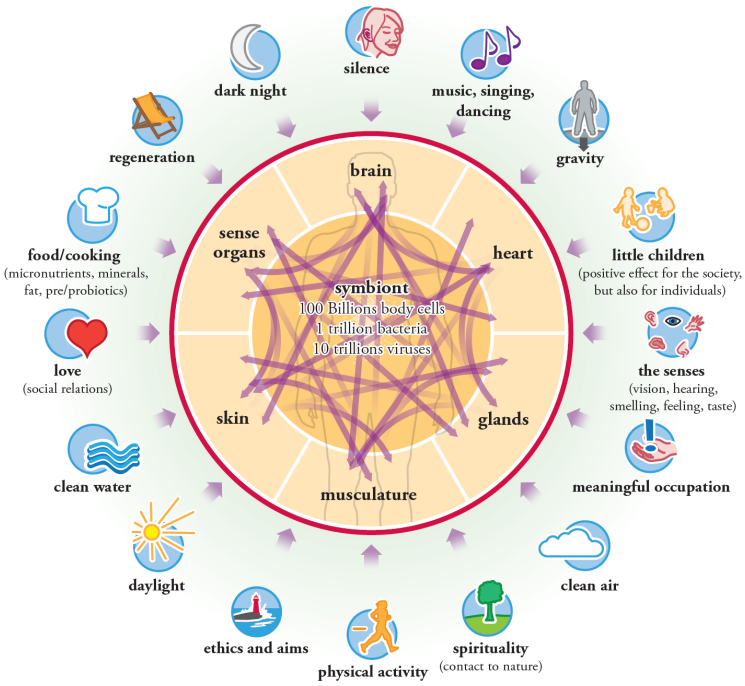

Fig 3: Complex interaction (ressources) of our environment with our body. Due to limited space not all factors of table 1 are depicted (copyright mip).

So there is no doubt that our lifestyle is highly responsible for the huge amount of chronic diseases by which the population of the civilized nations are affected. Instead of procuring the necessary ressources for our body as our natural environment has done formerly, the new technical world exposes the population to toxic chemicals and endocrine disruptors like aluminium and fluoride. Figure 3 gives an overview of the complex interaction of the symbiotic human body with the ressources of his environment.

Conclusion: Non-communicable, chronic diseases are the dominant health problem in the 21st century worldwide. Scientific effort should not only focus on avoiding negative factors like pesticides, fluoride, and aluminum, but should also press for greater attention to compensating factors in case of inevitable exposure. This can be done by remembering and restoring the natural resources mankind has lost so extensively as a result of technical progress and lifestyle changes.

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FLUORIDE REDUCES VITAMIN D AND GLUCOSE TOLERANCE IN SCHOOL CHILDREN

Anurag Tomar[1,2,6], Dushyant Singh Chauhan[1,3], Vivek Pratap Singh[1,3], Mukesh Tiwari[1,4], Swati Tomar[1,5], Sandeep Tripathi[1,3]

Abstract:

We aimed to determine the relationship between glucose intolerance and serum 25-hydroxyvitamin D (25-OHD) levels in fluoride (F) exposed school children in a rural area of Rajasthan, India. Seventy boys, aged 9–14 years, from a high F rural area, drinking water F>2.5 ppm, were compared to 70 healthy age-matched controls boys from a low-F rural area, drinking water F<1.5 ppm. Anthropomorphic data were collected from both the subject and control groups. Serum and urine F, fasting serum glucose, insulin, serum calcium, alanine aminotransaminase (ALT), and 25-OHD were measured. The concentration of 25-OHD in the subject group was significantly lower than that of the control group ($p<0.001$). Serum calcium and ALT were markedly elevated in the subjects compared to the controls ($p<0.001$). A correlation was found between vitamin D deficiency and glucose intolerance. In the high F group, glucose tolerance was affected by both high F exposure and deficient 25-OHD and the subjects were at risk of having bone deformities and hypoglycaemia.

Keywords: 25-OHD; Fluoride effects on ALT; Fluoride effects on bone; Fluoride effects on glucose tolerance; Fluoride effects on serum calcium; Fluoride effects on Vitamin D; Fluoride in drinking water; children; Hypoglycaemia; Vitamin D

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Platform Session 2

Fluoride and the renal, respiratory, and cardiovascular systems

Associate Professor
MASASHI TSUNODA

EXPERIMENTAL APPROACH TO THE TOXICITY OF FLUORIDE AMONG A SENSITIVE POPULATION: USE OF RODENTS WITH RENAL FUNCTION IMPAIRMENT

Masashi Tsunoda[1], Takamasa Kido[2], Mayuko Hosokawa[3]
Sagamihara, Japan

Introduction

Fluoride (F) widely exists in nature. The contamination of groundwater by F has been reported worldwide. Many inhabitants who drink well water with F at 8 ppm or more suffer from endemic fluorosis. In Japan, the levels of F in the groundwater are not high as 8 ppm, and the F concentration in tap water is regulated at under 0.8 ppm; therefore, osteofluorosis has rarely occurred. However, water fluoridation, which has not been introduced in Japan, is also controversial among Japanese societies. Considering water fluoridation, the sensitive people to F among the general population should be considered.

In the study used common mice as an experimental model exposed to F via the drinking water (Tsunoda, et al., 2005), the toxic effects of F were not clearly observed among the mice exposed to 125 ppm of F in their drinking water. Table 1 demonstrated the body weight and relative organ weights of mice exposed to F for 1 month via drinking water.

Group	Final body weight (g)	Liver (mg/g b.w.)	Kidney (mg/g b.w.)
Control	27.60±1.17	55.55±1.62	15.40±0.30
1 ppm F	26.93±0.35	55.10±1.17	15.29±0.28
5 ppm F	28.15±0.48	55.18±0.97	15.16±0.14
25 ppm F	28.20±0.46	55.74±1.53	14.91±0.31
125 ppm F	26.90±0.56	56.28±0.74	15.28±0.32

Table 1. The body weight and relative organ weights of BALB/c mice exposed to fluoride at 0, 1, 5, 25, 125 ppm in their drinking water (Tsunoda, et al., 2005)

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There were no significant differences among the groups. Figure 1 illustrates the analyses of dopamine and dopamine matabolites in the hypothalamus from the mice exposed to F via drinking water.

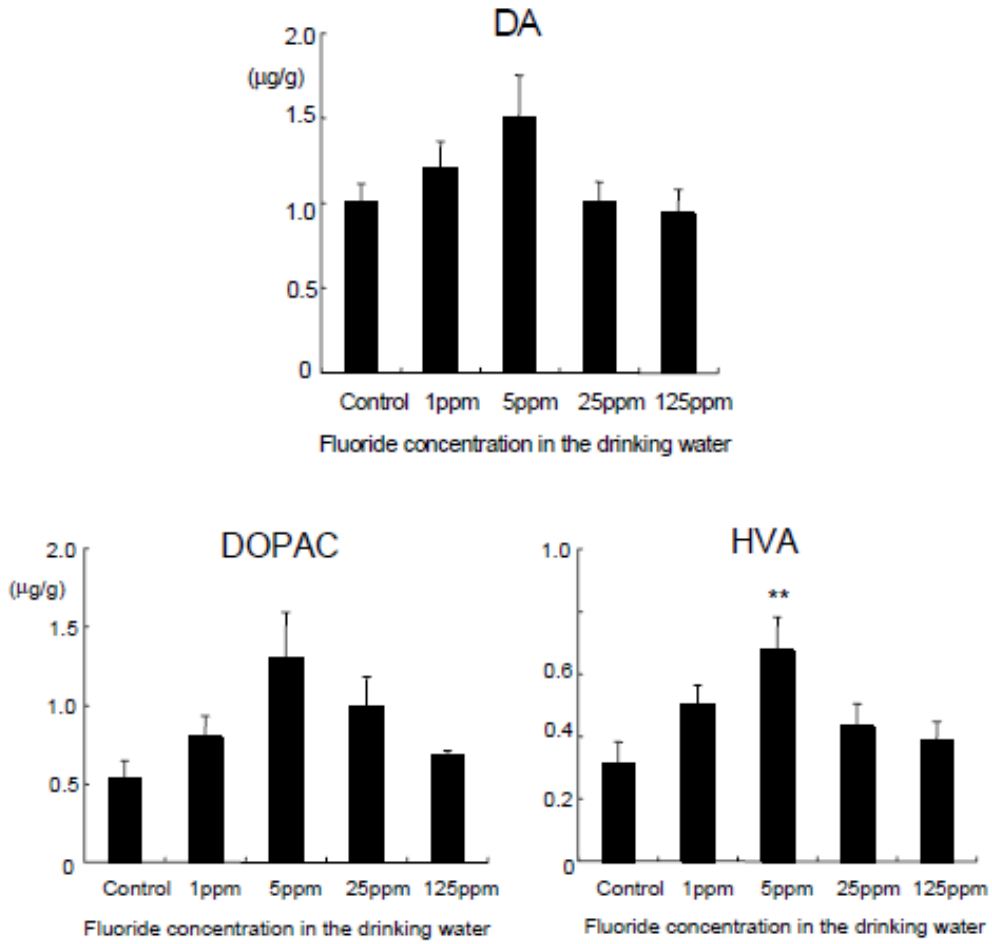

Figure 1. The levels of dopamine and dopamine metabolites in the hypothalamus from the BALB/c mice exposed to fluoride via drinking water at 0, 1, 5, 25 and 125 ppm for 1 month (Tsunoda, et al., 2005).

DA: dopamine, DOPAC: dihydroxyphenylacetic acid, HVA: homovanillic acid,
**: $P < 0.01$ by Fisher's PLSD test.

Although the alterations in dopamine and homovanillic acid in the hypothalamus among the groups were observed, those were not dose-dependent. As long as the use of animals with normal kidney function, it may be difficult to detect clear toxic effect induced by F via drinking water. Therefore, we focused to renal function.

F is filtered from the blood by the kidney and excreted in the urine. Renal function is quite

important and may be closely related to the differences of susceptibilities to F toxicity in the general population. F may accumulate more readily in individuals who have impaired renal function and, therefore, affects them more seriously. Also, the kidney is the target organ of F. To elucidate the effects of F on the population with impaired renal function, evidence using adequate animal models is required. In this keynote speech, we describe the previous studies for F toxicity using animal models with impaired renal function.

ICGN mice

ICR-derived glomerulonephritis (ICGN) mice spontaneously develop glomerulonephritis resulting in nephritic syndrome. We used such mice as an animal model with renal impairment. Hosokawa et al. (2010) administered F to ICGN mice at the ages of 11−14 weeks and ICR mice as controls with normal kidney function. Male ICGN mice were exposed to F at concentrations of 0, 25, 50, 100 and 150 ppm in their drinking water for 4 weeks. Figure 2 illustrates the viabilities of ICGN mice exposed to F. All of the ICGN mice exposed to F at 150 ppm died within 4 weeks with significant decreases in their body weight. Four of 9 ICGN mice died during the observation period. Whereas, no ICR mice exposed to 150 ppm died.

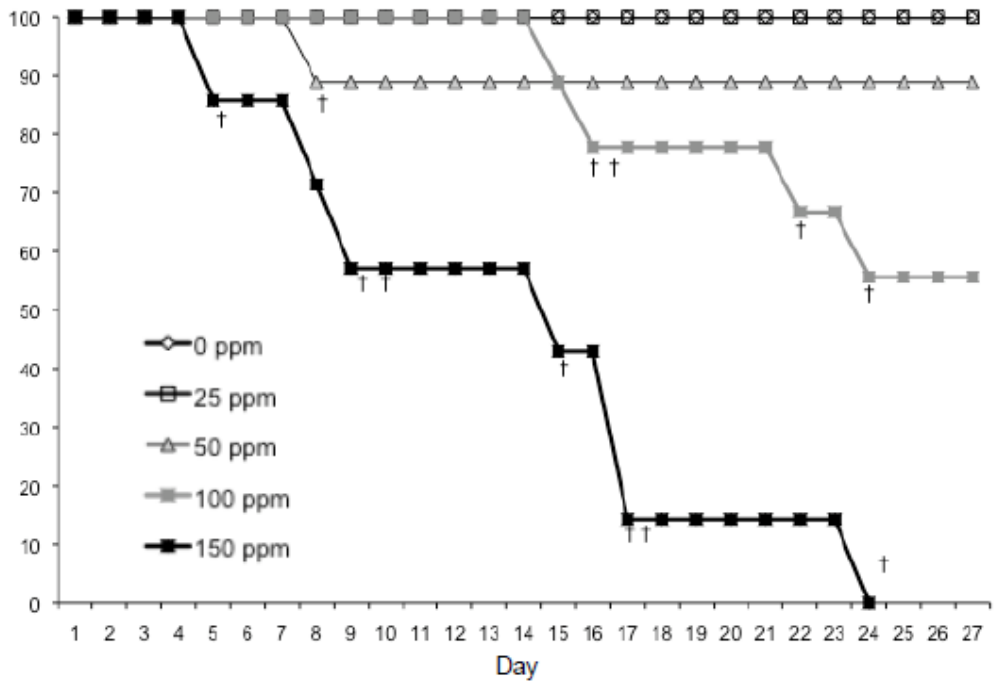

Figure 2. Viabilities of ICGN mice exposed to 0, 25, 50, 100 and 150 ppm F in their drinking water for 1 month (Hosokawa et al., 2010)

For the ICGN mice, the mean value of BUN in the 150-ppm group was significantly higher than those in all the other groups at the end of the observation period.

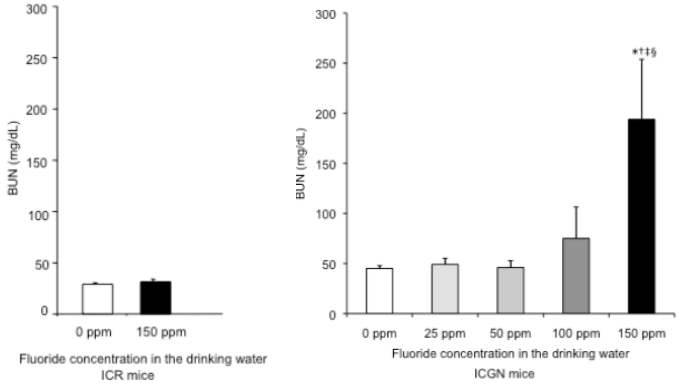

Figure 3. BUN values of ICGN mice and ICR mice exposed to fluoride for 1 month (Hosokawa et al. 2010)

For each mouse, the BUN values rapidly increased in most ICGN mice exposed to 150 ppm F before death. The mean value of serum creatinine in the 150-ppm-exposed ICGN mice was also significantly higher than those in all the other groups.

Figure 4 illustrates the final concentrations of F in the serum of ICGN mice and ICR mice after the exposure to F. Since all ICGN mice exposed to 150 ppm F died, there was no data of final mean serum F concentration for the group. The mean serum concentrations of F in the ICGN mice were increased with the doses of administration. The mean value of serum F in the ICGN mice exposed to 25 ppm at the end of the observation period was 0.43 mg/L, which was similar to the mean serum F concentration of 0.50 mg/L in the ICR mice exposed to 150 ppm.

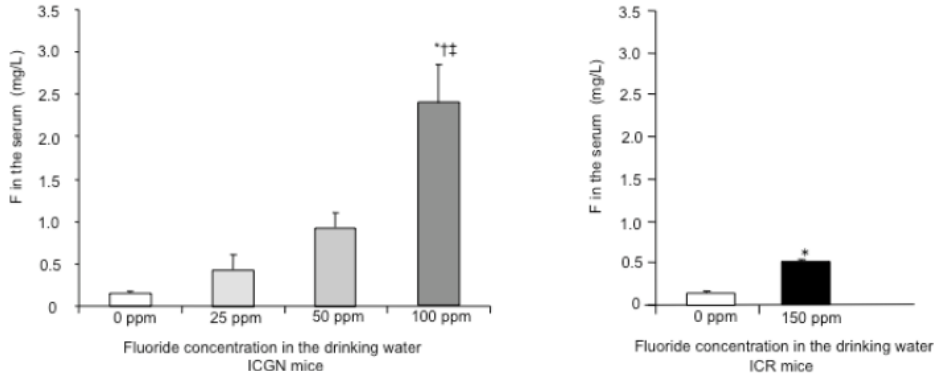

Figure 4. Fluoride concentrations in the serum of ICGN mice and ICR mice exposed to F at 0, 25, 50, 100 and 150 ppm in the drinking water (Hosokawa et al., 2010)

*: $P < 0.05$ compared with the control, †: $P < 0.05$ compared with the 25 ppm group, ‡: $P < 0.05$ by Student-Newman-Keuls test.

In the study by Hosokawa et al. (2011), the ICGN mice and ICR mice were exposed to F by the same protocol, and the renal histopathology of mice was examined. As same as the

result of Hosokawa et al. (2010), all of the ICGN mice exposed to F at 150 ppm died within 4 weeks. Therefore, the mice exposed to F at 150 ppm were not pathologically examined. Figure 5 illustrates renal histopathology of the ICGN mice exposed to F at 0, 25, and 100 ppm F in the drinking water. In the control ICGN mice, the glomerular capillary wall thickened slightly. In the 25-ppm ICGN mice, a slightly increased mesangial matrix and slight dilation of urinary tubules were observed. In the 100-ppm ICGN mice, thyroidization and a remarkable increase in the mesangial matrix were observed. The deterioration of glomerulonephritis by F were clearly demonstrated among the mice exposed to 100 ppm F.

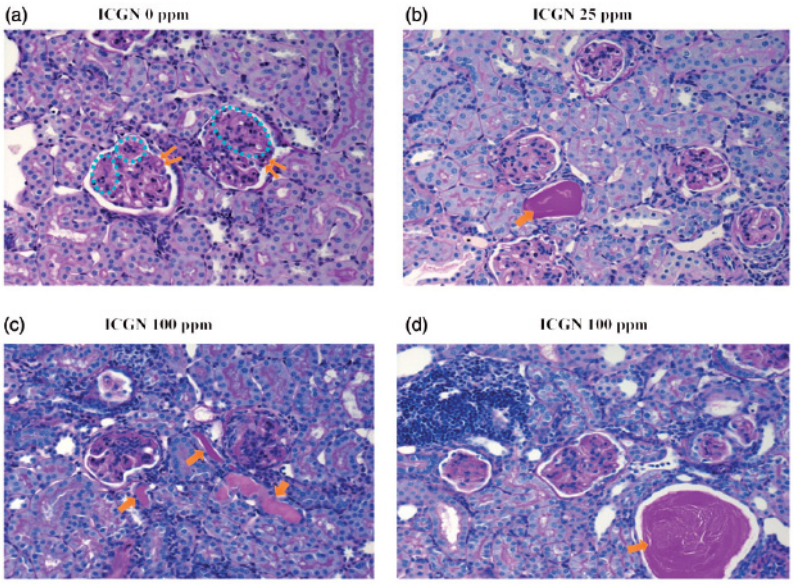

Figure 5. Renal histopathology of ICGN mice exposed to F at 0, 25 and 100 ppm in the drinking water for 1 month (Hosokawa, et al., 2011)

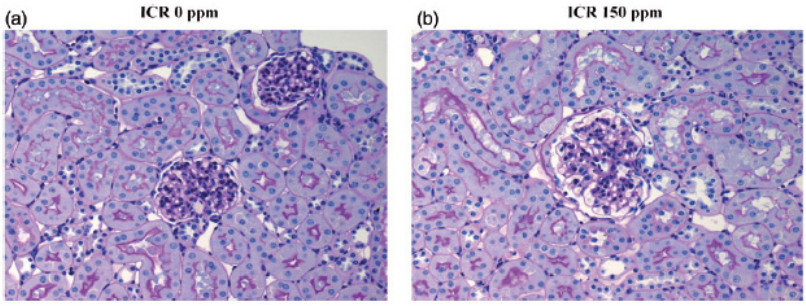

Figure 6. Renal histopathology of ICR mice exposed to F at 0 or 150 ppm in the drinking water for 1 month (Hosokawa et al. 2011)

However, there were no pathological changes in the glomeruli or urinary tubules of the ICR mice exposed to F at 0 or 150 ppm (Figure 6).

The immunotoxic effects by F were also suggested in the study. In the ICGN mice exposed to F at 150 ppm, the mean value of the relative spleen weights, which were determined at the

time of death of the mice, was significantly lower than that in the control.
These results clearly suggested that F exaggerated its toxicity in the mice with impaired renal function. It should be noted that the serum concentration of F in the ICGN mice exposed to F at 25 ppm was similar to that in the ICR mice exposed to 150 ppm. ICGN mice are good experimental models for a population with impaired renal function due to glomerulonephritis. The difficulty in using ICGN mice as an experimental model for renal failure is that they are not readily available. Commercially, pregnant ICR rats that have been transplanted with 50 frozen embryos of ICGN mice are provided, and it is necessary to breed them. Therefore, much manpower and resources are required.

HIGA mice

IgA nephritis is the most common chronic glomerulonephritis among Japanese. High IgA (HIGA) mice, a mutant of ddY mice, develop IgA nephropathy spontaneously. HIGA mice are commercially available. Kido et al. (2012) evaluated the toxic effects of F on HIGA mice. In the study, male HIGA mice, 11−12 weeks of age were exposed to F at concentrations 0, 50, 100, or 150 ppm in their drinking water for 4 weeks. Male BALB/c mice, with normal kidney function as controls, were also exposed to F at the same doses as the HIGA mice. The urine of each mouse was sampled for 24 hours once a week by using a metabolic cage. The concentrations of creatinine and protein in the urine were determined. No mice died in this study, and there were no significant differences in body weight among either the HIGA mice or the BALB/c mice. Significantly higher levels of urinary protein compared to those in the control were observed in the 100-ppm group of HIGA mice after 3 weeks from the beginning (Figure 7) and in the 100-ppm group of BALB/c mice after 2 weeks (Figure 8).

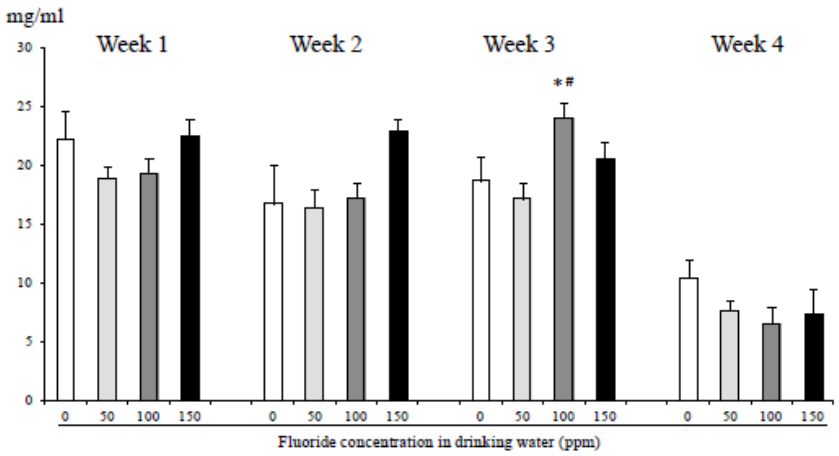

Figure 7. The protein levels in the urine of HIGA mice exposed to fluoride in their drinking water (Kido, et al., 2012)

*: $P < 0.05$ compared with the control, #: $P < 0.05$ compared with the 50 ppm group by Student-Newman-Keuls test.

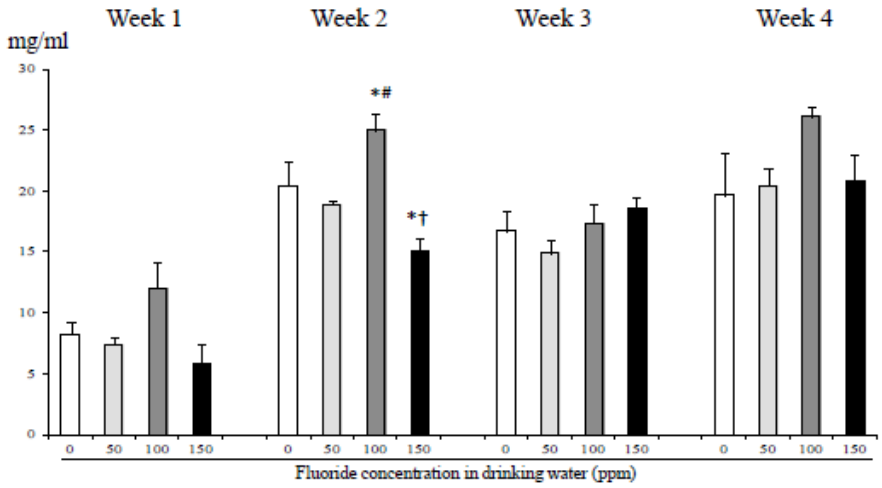

Figure 8. The protein levels in the urine of BALB/c mice exposed to fluoride in their drinking water (Kido, et al., 2012)

*: $P < 0.05$ compared with the control, #: $P < 0.05$ compared with the 50 ppm group, †: $P < 0.05$ compared with the 100 ppm group by Student-Newman-Keuls test.

The relationship between the protein level in the urine and the F dose was not dose-dependent, and these significant differences were observed just temporarily. For creatinine in the urine, the significantly lower mean values compared to those in the control were also observed temporarily.

Because the toxic effects of F on the HIGA mice were not clearly observed in the study, it was suggested that the HIGA mice at the ages of 11−12 weeks were not adequate as an animal model of impaired renal function. The IgA nephritis of HIGA mice may not advance further in 11−12 weeks. For a more adequate model of human patients with IgA nephritis, older HIGA mice should be used.

UUO rats

Unilateral ureteral obstruction (UUO) rats have been used as an experimental model of rats with impaired renal function. The tubrointerstitial fibrosis is observed in the UUO rats and their proximal tubular function is impaired. The good point of UUO rats is that with adequate surgical technique, unilateral ureteral obstruction is not difficult for common rats. Although it is difficult for the UUO rats to use them in the experiment with observation period more than 2 weeks, the UUO rats can be used for the evaluation of the toxicity of F for rats with impaired tubular function. It is better to have an experimental model of rats in addition to mice. In our preliminary study (Kido et al, 2013), male SD rats were treated with left UUO or sham-operation. F was administrated to the UUO rats at 0, 75 and 150 ppm and the sham rats at 0 and 150 ppm in the drinking water for 2 weeks. The mean body weights of the UUO rats

exposed to F at the concentration of 150 ppm in the drinking water were significantly lower than those of the controls (Figure 9).

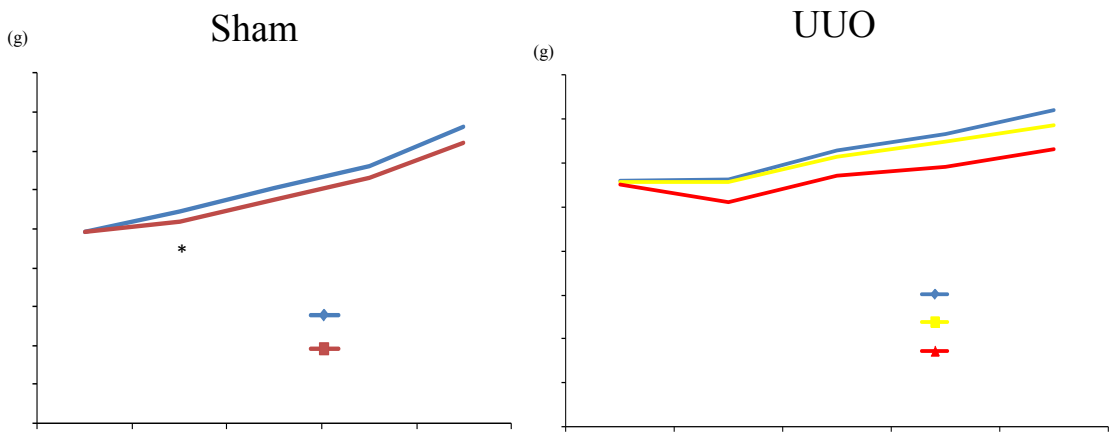

Figure 9. The alterations of body weights of the UUO rats and rats with sham operation exposed to fluoride via drinking water for two weeks (From the presentation by Kido, et al., 2013)
**: P <0.01 by Fisher's PLSD test.

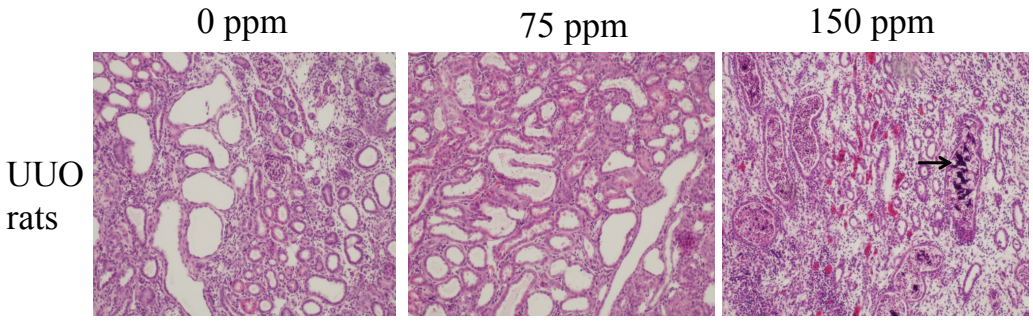

Figure 10. The typical images of the cortex of kidney from the UUO rats exposed to fluoride for 2 weeks (From the presentation by Kido et al., 2013).

From the pathological examination, it is suggested that tubulointerstitial fibrosis induced by UUO is deteriorated by the administration of F (Figure 10). From these results, it is suggested that the UUO rats are useful for the evaluation of F toxicity induced by the intake from drinking water.

Other studies and conclusions

Generally speaking, there are many factors that affect the sensitivities of hosts to toxicants. For example, F1-generation mice are relatively more sensitive to environmental pollutants such as methyl mercury than are adult mice. The toxic effects of F on F1-generation mice

also need to be studied.

For the evaluation of the toxic effects of F, the standpoint of considering a highly sensitive population, such as one with kidney function impairment, is essential. For that purpose, adequate animal models are useful.

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Declan Waugh

EPIDEMIOLOGY OF FLUORIDE AND ITS CONTRIBUTION TO INFLAMATORY RESPIRATORY DISEASE.

Declan Waugh, September 2013

Abstract

This study aims to explain the international differences in prevalence rates of inflammatory respiratory disease, in particular asthma and the role fluoride exposure plays in the pathology of the disease and reported burden of disease. Asthma is a major public health concern and among the leading causes of disability worldwide. The populations throughout the world where the prevalence of asthma are highest share one common environmental exposure risk factor. Throughout the world, across all regions, in every country, the highest burdens of these diseases are consistently to be found where the population is exposed to elevated anthropogenic or naturally occurring sources of fluoride. The highest burdens of disease however, are to be found in countries with artificially fluoridated drinking water.

This supports the available evidence on the comparative toxicity of fluoride compounds and suggests a direct causal role in the pathology of inflammatory diseases. This study presents the epidemiological evidence worldwide from both artificially fluoridated and non-fluoridated countries, areas where there are high naturally occurring fluoride levels in drinking water, as well as geographic areas with high exposure to atmospheric fluorides from coal combustion or other industrial sources. This study presents overwhelming epidemiological evidence demonstrating a direct causal relationship between dietary exposure to fluoride and inflammatory respiratory diseases. The same geographic pattern of disease burdens have also been identified for other inflammatory diseases; however this study only examines the evidence for asthma.

This study provides important data on the contribution of anthropogenic and naturally occurring fluoride to ill-health and mortality from disease. It aids in understanding the variation in prevalence and risk factors in disease burdens worldwide. Furthermore, given the acknowledged role of inflammation in the pathogenesis of cardiovascular disease, diabetes, rheumatoid arthritis, Alzheimer's, autoimmune diseases, and cancers, it may be safe to assume that increased fluoride exposure may be a contributory factor to the higher prevalence of these diseases in fluoridated countries. The study highlights the critical importance and urgent need

for proper health risk assessment, due diligence, monitoring, evaluation, and review of fluoridation policies by public authorities in support of assuring the health, safety, and economic well-being of citizens.

The summary information in this conference paper forms part of a much larger review study that is to be published shortly in the journal *Fluoride,* the quarterly journal of the International Society for Fluoride Research.

INTRODUCTION

Fluorine is the most reactive of all halogens. The elemental form of fluorine, is so chemically reactive that it rarely occurs naturally in the elemental state. Fluoride is the ionic form of fluorine. Fluoride is not an essential element for human growth and development, and for most organisms in the environment.[1]

Fluoride (either as hydrogen fluoride, particulate fluorides, or fluorine gas) is released to the atmosphere by natural sources such as volcanoes and by a number of anthropogenic sources particularly coal burning, steel manufacturing, mining, aluminium processing, fertiliser production and in the manufacture of glass, steel, cement, ceramics, pharmaceuticals, as an active ingredient in pesticides, herbicides fumigants and detergents. Fluoride is also used in the nuclear industry, in the manufacture of semiconductors and in energy storage. Another industrial application is as a surfactant in the emulsion of polymerization of fluoropolymers in the manufacture of prominent consumer goods such as Teflon and Gore-Tex. One of the most common medical uses of fluorine is as a chloroflouro carbon gas in asthmatic inhalers.

Fluoridation of public water supplies began in the United States of America post 1945 as a measure for the prevention and control of dental caries in children. The policy was also subsequently implemented in a number of other largely English speaking countries including Australia, the Republic of Ireland, Canada, New Zealand and the United Kingdom. Artificial fluoridation of public water remains a controversial practice, with a number of countries ending the practice in the 1960, 70s and 80s including the Netherlands, Sweden, Finland, Germany, the Czech Republic, China and Japan. In 2013, Israel introduced legislation ending the policy of mandatory fluoridation. Since the late 1960s fluorides have also been an active ingredient in toothpaste and fluoridation of salt for consumption has recently been advanced in some countries, particularly Central and South America, the Caribbean, Switzerland and Germany.

Fluorides are known to have wide ranging adverse effects on the environment, human and animal health. For over a century inorganic fluoride emissions have damaged crops, forests, and natural vegetation, and they caused fluorosis in factory workers, livestock and wild mammals. There is no doubt that inorganic fluoride was one of the major pollutants of the 20th century. [2] Emissions and exposure to fluorides increased dramatically post World War II following rapid industrialization in some economies. Apart from geographic regions of the world where fluorides in high concentrations are found naturally in groundwater, such as India and China,

the increase in human exposure to fluoride was most profound in countries that commenced artificial fluoridation of drinking water supplies. However, the recent rapid industrialization of China, as well as India, supported by their massively increased consumption of coal and industrial emissions has resulted in an increase fluoride exposure of their populations. In certain countries the increased abstraction of groundwater as a source of drinking water has also resulted in significant increased exposure of the population to fluoride.

In general, however, the major sources of human exposure to fluoride are drinking water, food, dental products, and pesticides. In countries that practice artificial fluoridation of water the largest contributor is water, in countries that practice fluoridation of salt, the largest contributor is food. In some instances population exposure to industrial fluoride emissions can be a significant contributor to their total intake of fluoride.

The long term health effects of exposure to fluoride remain to be comprehensively studied. This was addressed in the ground-breaking report published by the U.S. National Academies of Sciences in 2006.[3] In this paper, I have concentrated on just one of the many human health effects arising from exposure to fluorides, addressing inflammatory respiratory disease, which represents one of the most significant and rapidly growing chronic disease burdens present in certain countries today.

This research examines industrial and anthropogenic sources of fluoride exposure and published epidemiology data on global inflammatory respiratory disease burdens. The study examines how increased exposure to fluoride, particularly from artificial fluoridation of water or salt, may have contributed to the dramatic and unparalleled increase in inflammatory disease, documented in certain geographic regions of the world in recent decades. Case studies and examples will be provided in the presentation to support the information in this paper.

Chronic respiratory diseases of the lungs represent a wide array of serious diseases. Preventable chronic respiratory diseases include asthma and respiratory allergies, (COPD), occupational lung diseases, sleep apnea syndrome, and pulmonary hypertension. They constitute a serious public health problem in all countries throughout the world, most especially in countries where artificial fluoridation of water and salt is a public health policy.

It has been demonstrated that exposure to a mix of allergens and irritants can at times promote the development of the disease (Gilmour et al 2006).[4] It has also been determined that exposure to fluoride augments the inflammatory response to irritants, and is thus a clear risk factor in the development of inflammatory respiratory disease. Research has revealed the importance of inflammation of the airways in asthma and the need for clinical treatment to reduce chronic inflammation. Yet surprisingly, no study, to my knowledge, has examined the effect of increased dietary exposure to fluoride, from fluoridated salt and drinking water, or from high levels of naturally occurring water-borne fluoride, and its contribution to inflammatory respiratory diseases. This is the first report to comprehensively investigate this public health problem.

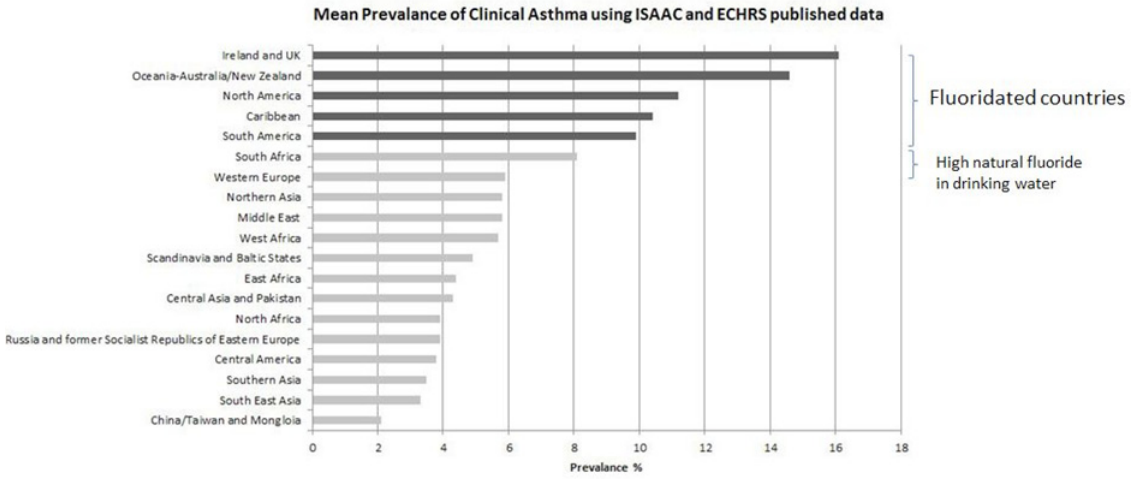
The International Study of Asthma and Allergies in Childhood (ISAAC) Phase One (1993-1997) examined the prevalence of asthma symptoms in 56 countries internationally and reported marked variations in the prevalence of asthma symptoms with up to 15-fold differences between countries. The highest prevalence of asthma symptoms were to be found in English-speaking countries (North America, Ireland, UK, Australia, New Zealand) and Latin America (Brazil, Peru, Argentina, Chile, Costa Rica, Jamaica, Uruguay). The report concluded that the major differences between populations found are likely to be due to environmental factors.[5]

The only other international survey of asthma comparable to the ISAAC is the European Community Respiratory Health Survey (ECRHS, 1996) which studied asthma prevalence's among adult males and females aged 20–44 years of age in 22 countries.[6,7] The ECRHS study, while predominantly focused on asthma prevalence among adults in Europe, did however include international centres in New Zealand, Australia, India, Algeria and the USA.[8] Of the 48 international centres that participated in the study, the highest prevalence of asthma symptoms was reported in Dublin, Republic of Ireland (RoI) with 32% of adults reporting wheeze symptoms. The RoI is the only European country with a national drinking water fluoridation programme.

The highest prevalence among the four centres in New Zealand was reported in fluoridated Wellington, at 27.3%. Fluoridated Melbourne represented the only centre in Australia, and reported a prevalence of 28.8%. The only centre included for the USA was Portland, Oregon, which reported a 25.7% prevalence of wheeze among the adult study group. While fluoridation of drinking water is extensively practised in Oregon, as in other US states, Portland remains the largest non-fluoridated city in the USA. The results for Portland while high by European standards are not representative of the USA in general, as far higher asthma prevalence's have been reported among fluoridated regions of the USA. This is discussed elsewhere in this report.

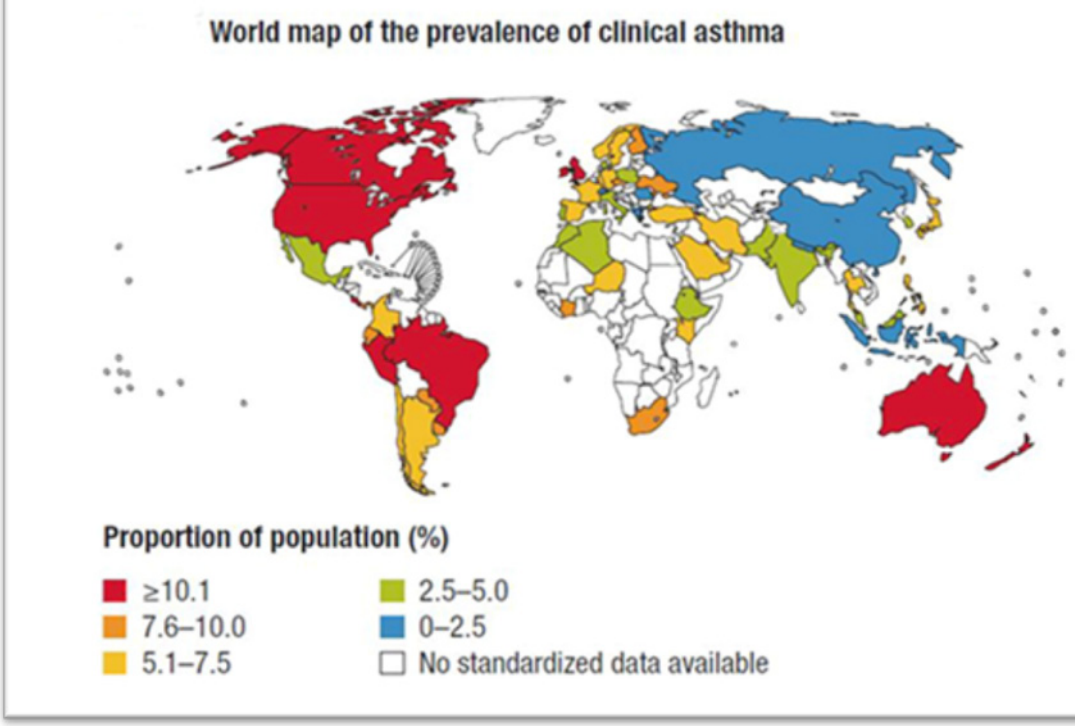
Among the 13 centres in 10 countries that were reported in both ECRHS studies, the ranking of prevalence of wheeze in the last 12 months was similar, with the fluoridated English-speaking countries (Australia, New Zealand, Republic of Ireland and the UK) having the highest rates, and non-fluoridated and Italy and Greece the lowest rates.[9]

The sites throughout the world where the prevalence of asthma is highest share one common environmental factor that can easily be identified--artificial fluoridation of drinking water supplies or salt fluoridation. This suggests that the recent "epidemic" of asthma in these countries may be directly related to exposure to anthropogenic sources of fluoride

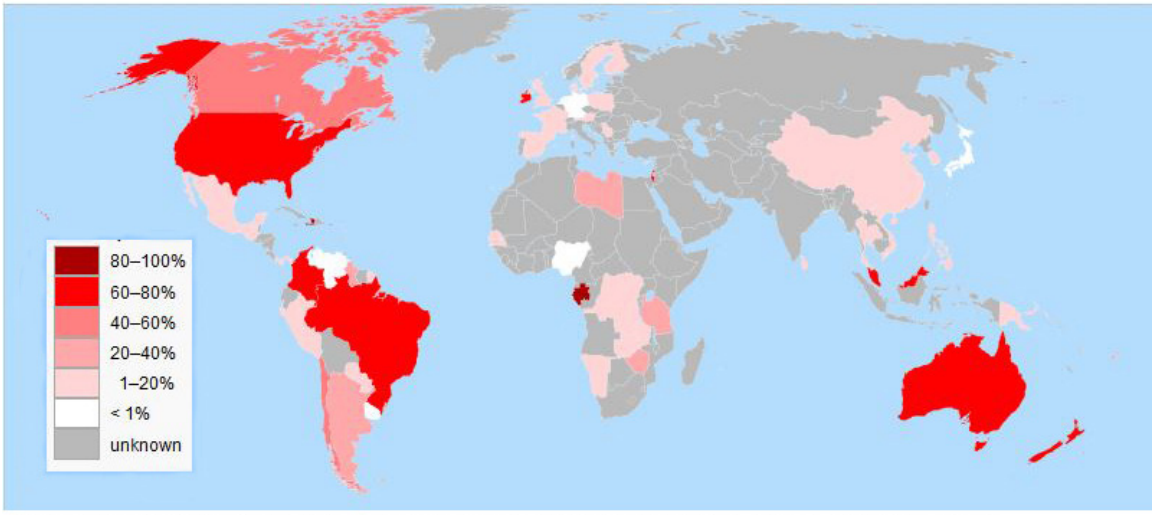


Source of Data:International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Health Survey (ECHRS) and Masoli et al. Global Burden of Asthma, Development for the Global Initiative for Asthma, Medical Research Institute of New Zealand & University of Southampton

The world map of the prevalence of asthma is based on these the ECRHS and ISAAC studies combined. The map clearly identifies that the highest prevalence worldwide of asthma is to be found in fluoridated countries including Australia, New Zealand, United States, Canada, Brazil and Ireland.



Percentage of population receiving fluoridated water, including both artificial and natural fluoridation



Source: Sellers C. The artificial nature of fluoridated water: between nations, knowledge, and material flows. Osiris. 2004;19:182–200

The countries with the highest burdens of inflammatory respiratory disease significantly above the global average include the Republic of Ireland, the Unites States of America, Australia, New Zealand, Canada, and Brazil, all countries with artificial water fluoridation, and Peru, where there is a mandatory national salt fluoridation programme. Recent published evidence, from peer reviewed journals as provided in this report, clearly supports a direct causal relationship between increased population exposure to fluorides asthma, COPD and other inflammatory diseases. This relationship appears more aggressive among populations exposed to artficially fluoridated water, reflecting the nature and toxicity of fluorosilicates in comparison to naturally occuring fluorides in the environment.

The ISAAC reported that a striking feature of international studies on asthma prevalence is the high rates of asthma symptoms in countries whose predominant language is English.[10] The ISAAC however neglected to mention that the one commonality between these countries was fluoridation of water.

The ISAAC also reported that high rates of asthma symptoms were also found in some non-English-speaking countries. These countries are also fluoridated, but asthma rates were not uniform among countries sharing the same language.[11] For example, the authors reported that Peru and Costa Rica had a much higher prevalence than Spain, Brazil had a higher prevalence than Portugal, and Hong Kong had a higher prevalence than China. Once again the common risk factor is that Brazil, Peru, Costa Rica, and Hong Kong have water or salt fluoridation, while Portugal, Spain, and China do not.

Fluoridation of public water began in the United States in 1945; currently only eleven countries in the world have more than 50% of their populations drinking artificially fluoridated

water (Australia, Brunei, Chile, Hong Kong, Ireland, Israel, Guyana, Malaysia, New Zealand, Singapore, and the United States). In 2013, Israel passed legislation to end mandatory fluoridation in 2014. Within Europe only the Republic of Ireland has a national legislative policy mandating artificial fluoridation of all public water supplies. Fluoridation of water is undertaken in a few regional locations in Spain and the UK, where approximately 10% of the respective countries populations are provided with fluoridated drinking water.

The rapid increase in asthma in fluoridated countries such as the USA, Brazil, Canada, Australia, Ireland, and New Zealand, as well as in countries with salt fluoridation programmes, such as Mexico, Jamaica, Costa Rica, Peru, and Chile, has undeniably occurred in parallel with increased dietary exposure to fluorides, either through water or fluoridated salt (FS).

The United States National Research Council (NRC, 2006) reported that there can be no question that fluoride can affect the cells involved in providing immune responses, and that fluoride exposure increases the inflammatory response to irritants. It was hypothesized that the main route was by means of activation of G-protein complex.[12] Consequently, it is entirely plausible that increased or early exposure to fluorides and silicofluorides from artificial fluoridation chemicals may have significant implications for inflammatory diseases--in particular, for diseases of the respiratory system.

Perhaps the best evidence to support fluoride's contribution to respiratory diseases comes from Mexico and Israel.

Mexico commenced mandatory national salt fluoridation in 1995, while Israel commenced water fluoridation in 1981. Official Mexican Department of Health figures recorded that immediately after commencement of their national salt fluoridation programme the number of new cases of asthma increased fivefold within a year, and the incidence of acute respiratory infections doubled. [13]

Likewise in Israel, following commencement of water fluoridation, asthma prevalence rates increased significantly. Sacher et al. (1994) reported that between 1980 and 1991, asthma prevalence among 17±18 year olds increased by 85% among men and 115% among women.[14]

In Spain, the highest prevalence of asthma and respiratory disease are also to be found in fluoridated communities, particularly in the Basque province. The ISAAC (1998) reported the prevalence of 'asthma ever' among those 13-14 years old in the Basque city of Bilbao at 16.3%, compared to a national average of 10.5%.[15] High levels, significantly above the national average are also reported for adults in Seville.[16] Seville is the largest city in southern Spain that is fluoridated.

The only regions in Spain with comparable burdens of respiratory disease are located in geographic areas with high exposure to anthropogenic fluorides from phosphate mining, fertilizer manufacture, coal power stations and aluminum smelting.

Similarly in the UK, the highest burdens of respiratory disease are to be found in England within communities provided with artificially fluoridated drinking water. This is especially

evident in parts of the East and West Midlands, as well as North East England which together represent the geographic regions that have been the longest fluoridated in England.

Since commencement of salt fluoridation in Germany, recent epidemiological data also demonstrates a significant increase in asthma prevalence. [17] Very significant increases have also been reported in Switzerland, where asthma prevalence has increased fivefold since a national salt fluoridation programme commenced. [18] Currently approximately 8% of the Swiss population suffers from asthma, as against only 2% some 25-30 years ago. [19]

In France, the market share of FS has declined dramatically from over 60% of all salt sold to less than 10% in recent years. In the region of France with the highest use of fluoridated salt, Montpellier, where FS is 70% of market share, and where FS is also used in public school canteens, the prevalence of childhood asthma is more than 50% above the national mean.[20]

In the Republic of Ireland, asthma prevalence has increased fivefold since the period when fluoridation of drinking water commenced. Currently, despite some of the best ambient air quality in the world, the prevalence of childhood and adult asthma in Ireland is now amongst the worst in the world and far greater than any other European country.

Although there is no artificial fluoridation of drinking water or fluoridation of salt in any of the Scandinavian countries, they do report variable levels of naturally occurring fluoride at relatively high levels in drinking water. The lowest prevalence of asthma in Finland, Norway, Sweden and Denmark are to be found in regions with the lowest level of fluoride in water.

The prevalence of asthma in the U.S. has a marked geographic variation, the highest prevalence's are to be found in the regions with the greatest penetration of fluoridation of drinking water supplies including the District of Colombia, the State of Kentucky and Maryland or regions with naturally elevated fluoride levels (4-7ppm) in drinking water such as North Dakota. A pattern of disease prevalence associated with water chemistry is also evident. States with very soft water that are artificially fluoridated such as Maine, New York, Vermont, Delaware and Michigan have reported high prevalence's of asthma. The highest rates of lung cancer are also to be found in the states with low calcium fluoridated water.

Similarly, a marked geographic variation in asthma prevalence is observed in Canada, with the lowest prevalence of childhood asthma recorded in non-fluoridated provinces such as British Colombia (14.4%) compared to much higher disease burdens in fluoridated provinces such as Nova Scotia, Newfoundland, New Brunswick and Prince Edward Island where rates are significantly higher at 21.7%.[21]

Fluoridation exists in three forms in South and Central America. Water fluoridation is practiced in Brazil and Chile with lesser programmes in Argentina and Peru. National salt fluoridation programmes are implemented in Costa Rica, Peru, Mexico, Uruguay, Bolivia, Colombia, Jamaica, Ecuador and Cuba. Milk fluoridation is also practised in parts of Chile. The first countries to fluoridate refined domestic salt in the Americas were Jamaica and Costa Rica (1986–1987).[22] Mexico became the seventh country to implement a salt fluoridation

programme in 1991.

Marthaler (2005) reported that in the period 2004-2005 100% of the population of Jamaica was consuming fluoridated salt and 95% of the population of Costa Rica. Mexico approached 90-95%, Uruguay 90%, Colombia 80% and Ecuador 80%.[23]

In the Americas, excessive levels of salt intake are well documented as such salt fluoridation has a significant role to play in dietary fluoride intakes and can be a significant contributor to dental fluorosis.Recent epidemiological data f from Mexico and Costa Rica revealed that the prevalence of dental fluorosis in areas where fluoridated salt is used.[24], [25]

Today, Brazil is one of most extensively fluoridated countries in the world with 73 million people in over 3350 communities provided with artificially fluoridated water. Brazil also has one of the highest prevalence's of asthma globally[26] Among the general population, the prevalence of clinical asthma is reported at 11.4%. The ISAAC (1998) reported the mean prevalence of asthma ever among 13-14yrs olds at 14.9% with much higher prevalence's in Porto Alegre (21.9%) and Recife (20.9%). The Porto Alegre region, Southern Brazil has a natural fluoride level in water of up to 6.13mg/L, while Recife is the fifth-largest metropolitan area in Brazil and is drinking water is artificially fluoridated. The lowest prevalence of 'asthma ever' recorded in the among the participating cities in the ISAAC study was in Curitba at 8.6%. Curitba is non fluoridated.

In other fluoridated countries such as Chile the incidence of childhood asthma almost doubled between 1994 and 2002, with over 15% of children aged 13-14years diagnosed with respiratory disorders. [27]

A very high incidence of dental fluorosis[28] and asthma[29] has also been reported in Peru. For the period 2004, acute respiratory infections for both males and females in children and adults, were the leading cause of death in Peru followed by ischemic heart disease.[30]

In Colombia, a country with a universal mandatory salt fluoridation programme, the incidence of asthma is very high. Recent epidemiological data reported that the overall lifetime accumulated prevalence of asthma symptoms was 23% with asthma symptoms being the most frequent medical cause for visiting a medical physician in 2009.[31]

Costa Rica was one of the first countries to implement a national salt fluoridation programme in 1986-87. [32] Lai et al (2008) reporting on the ISAAC Phase three study reported that the prevalence of wheeze in the past 12 month in 13-14 year olds Costa Rican 6-7 year olds was 37.6%. The prevalence of severe asthma was 16% for 13–14 year olds and 20.3% in 6–7 year olds.[33]

Jamaica along with Costa Rica were the first countries to implement a national salt fluoridation programme. A recent study by Kahwa et al (2012) reported that almost a fifth (19.6%) of Jamaican children aged 2–17 years had current wheeze, while 16.7% had self-reported doctor-diagnosed asthma.[34]Kahwa et al(2010) reported that asthma was a significant public problem in the Caribbean with asthma prevalence of 20.4% of adults aged 18-24 had asthma

and 25.6% of the age group 25-34 compared to 21.6% of 35-44 year olds, 13.5% of 45-54 year olds and 8.65 of 55-64 year olds. For infants 2-4 yrs of age a prevalence of 18.6% was reported and 33% for children aged 5-9years of age.[35]

The highest prevalence's of respiratory disease in the Pacific and South Asia are to be found in countries with large scale artificial water fluoridation programmes and in the volcanic islands and low lying coral atolls of Caroline islands.

New Zealand has one of the highest reported prevalence's of asthma in the world[36], with 25% of children aged 6-7 years and 30% of 13-14yr olds with asthma symptoms in one recent survey[37]. In the fluoridated city of Dunedin an astonishing 35% of the children and young adults have been diagnosed with asthma.[38]

In Australia, the hospitalisation rates for asthma have more than doubled over the last 30 years. [39] Currently one child in six under the age of 16years is diagnosed with asthma in Australia.[40] This increase has occurred in parallel with increasing fluoridation of public water supplies. It has been also reported that children born in Australia have about a two-fold greater rate of asthma than those living in Australia but born elsewhere.[41]

Asthma & bronchitis are also major public health burdens in India. These chronic diseases are more predominant in children and aged population and are the leading cause of morbidity among male as and females in rural areas of India.[42] Excessive fluoride concentration in drinking water are also a serious public health problem in India.[43] It has been established that about 45% of drinking water sources in India are contaminated by fluoride.[44] The geographic areas worst affected are in the North South and East of the Country. Recent reports also show a wide variation (4–19%) in the prevalence of asthma in school-going children from different geographic areas in India.[45] The prevalence of asthma, as noted in other countries follows the same pattern of increased prevalence with increased exposure to fluoride.

Endemic fluorosis is also prevalent in China, covering 29 provinces, municipalities and autonomous regions. Today, however, the main source of fluoride exposure is from coal burning, inhalation of airborne fluorides and ingestion of fluoride contaminated foods. Research into fluoride emissions from coal-combustion systems is becoming increasingly important as the issue of fluoride pollution in China becomes more serious.[46] Endemic fluorosis related to coal combustion is a disease that has affected around 35 million people in China, and represents a serious health concern. In southwest China, fluoride from coal combustion has polluted the atmosphere and has impacted negatively on the temporal and geographical distribution of terrestrial vegetation.[47] People living in these areas are affected by chronic endemic fluorosis due to the excessive uptake of fluoride from air or food dried by coal-burning.[48],[49],[50] Other provinces such as Chongquing, in North East China are also badly affected.

While there are no nationwide figures for asthma in China, Chinese specialists estimated that the number of asthma cases they see has risen by about 40% between 2001-2005. [51] Prior to the recent economic development in China the prevalence of asthma and asthma related

symptoms was generally regarded as lower than western countries. Between 1990 and 2000 it was also reported that the prevalence of asthma among urban children rose 64%. In larger cities the increase was greater than 100%. The primary cause of the increase is undoubtedly air pollution resulting from the country's recent economic development. [52] This is reflected in the fact that respiratory diseases are the fourth most common cause of death after cancer, heart disease and stroke, which together account for over 80 per cent of deaths countrywide. Lung cancer is now the most common cancer in China. Deaths from this typically fatal disease have shot up nearly fivefold since the 1970s. [53] It is estimated that 26 per cent of all current deaths in China are caused by respiratory illnesses (compared with 2 or 3 per cent in the U.S.). In rural areas, respiratory disease is the number one killer. [54]

Among all of the published international studies on asthma however, the highest documented burden of the disease is to be found on inhabited islands of volcanic origin. This includes the Canary Islands, in the Atlantic, the Caroline Islands in the Western Pacific, and Tristan da Cunha, another volcanic island located in the South Atlantic Ocean. Very high levels of fluoride have been measured in drinking water in the Canary Islands, due to their volcanic geology. Correns (1956) reported fluoride levels in volcanic rocks of 80-2500ppm, while basalt rocks have been reported to contain up to 1000ppm fluoride. Similar very high asthma prevalence has been found in the Caroline Islands. The Caroline Islands are volcanic islands and low-lying coral atolls in the Southwestern Pacific, where asthma prevalence's of 51.4% and 41.9% have been documented.[55] The highest prevalence of asthma internationally, however, has been reported for Tristan da Cunha, with a rate of of 56%.[56]

Worldwide, data on prevalence of respiratory disorders provide compelling evidence that increased fluoride exposure has a causal relationship to increased prevalence of both asthma and COPD in countries that practice either extensive artificial water fluoridation or salt fluoridation. The countries with the most extensive programmes of fluoridation, and largest populations exposed to fluoride, clearly have the greatest prevalence of asthma in children and adolescents as well as within the older population. The burden of disease is, however, more focused on the younger populations. The youth have greater prenatal and infant exposure to fluorides than any previous generations and are at highest risk from the toxicity of fluorides, owing to their increased biological requirements for calcium to build developing bones. Fluoride is a calcium-chelating or -binding agent. Industrial synthetic fluorides are fully soluble and are all toxic calcium chelators.[57] The NRC (2006) reported that fluoride exposure has an indirect action of decreasing calcium absorption from the gastrointestinal tract and increasing bone formation; both of these effects lead to an increase in the body's calcium requirement. This perhaps is most pronounced in bottle fed infants fed formula milk constituted with fluoridated tap water. where the exposure to fluoride is up to 180 times (18,000 per cent) higher than for breast fed infants (0.7ppm versus 0.004ppm). It is acknowledged that fluoride formula fed infants have a significantly greater risk of developing dental fluorosis, which is a visible

sign of chronic fluoride overexposure.[58, 59, 60] Malnutrition and hungry will also significantly increase the bioavailability and toxicity of fluoride, especially in children.

As with fluoridation of water, increased fluoride intake from salt fluoridation may be a potential environmental factor for children, adolescents, and adults in the development of inflamatory respiratory disease. FS has already been conclusively shown to contribute to chronic fluoride intoxication in the form of dental fluorosis.[61,62] Both the ISAAC and ECRHS studies reported that the differences in prevalence of asthma in geographic regions internationally and within specific countries may be attributable to differences in environmental exposure. This study supports that view. There is little doubt in my mind, based on the available evidence that artificial fluoridation of water and salt has significantly contributed to the alarming increase in asthma and respiratory diseases. This increase has been most noticeable in the past two decades, particularly in Central and South America, in North America, Western Europe, Israel, and Oceania. What has further contributed to the burden of disease, especially in developing countries, as well as in lower socioeconomic populations in developed countries, is poor nutrition, which has been well documented to increase the toxicity of fluoride.[63,64,65,66]

The burden of asthma has wide ranging implications for health in general, not only in treating the disease itself, but its contribution to other chronic diseases. A recent study by Iribarren et al. (2012) reported on the association between adult asthma and risk of coronary heart disease, cerebrovascular disease and heart failure.[67] Their research found after adjustment for age, sex, race/ethnicity, cardiac risk factors, and comorbid allergy, asthma was associated with a 1.40-fold increased hazard of coronary heart disease, a 1.20-fold hazard of cerebrovascular disease, a 2.14-fold hazard of heart failure, and a 3.28-fold hazard of all-cause mortality. Stronger associations were noted among women. Remarkably their study reported that only asthma patients using asthma medications (particularly those on oral corticosteroids alone or in combination) were at enhanced risk of CVD. As noted previously, CFCs are used as propellants in inhalers in the administration of the drug. Tens of millions of patients use these metered dose inhalers on a daily basis, yet, no published research is available examining the long term health impacts of inhaling CFC gases and their potential contribution to total fluoride body burden or inflammatory disease. Tressaud (2006) warned that "the ignorance about the long term environmental effects of chlorofluorocarbon gases until the mid 1970s, at a time when their abundance was rapidly rising is a lesson which should forever be heeded"[68] When it comes to fluoride, it is evident that no public health authority in any fluoridated country has bothered to investigate in any detail its long term health effects, despite the comprehensive recommendations for extensive research to fill the current knowledge gaps as addressed by the NRC in their 2006 report.

Concluding Remarks

In conclusion, it is evident based on data from thirty three countries reported in this study that

exposure to fluoride through either ingestion via water or salt fluoridation or inhalation of airborne fluorides, has resulted without exception to significantly increased burden of respiratory disease. The reported increase prevalence of disease has however been most dramatic in countries with artificial fluoridation programmes suggesting increased human toxicity of synthesized chemicals used for the purpose of artificial fluoridation of water, compared to naturally occurring fluoride levels in water, even at much higher levels of population exposure. Dramatic and significant Increases in inflammatory respiratory disease burdens have been observed in ever country that has commenced programmes of artificial fluoridation of water, as well as fluoridation of salt. The reported increases in disease prevalence have been far higher than global averages and for non-fluoridated countries. The increase in the burden of inflammatory respiratory disease is of particular significance because most of it is human-induced, through increased exposure to environmental toxins such as synthetized fluoride chemical reagents, as well as industrial pollutants, and the increase in disease burdens in fluoridated countries has proceeded at a rate that is unprecedented in the human history.

While the burden of fluoride intoxication has affected all socio economic groups, the impact has been most severe on the lower income populations, most likely due to poorer nutritional status, increased prevalence of bottle feeding and greater consumption of fluoridated drinking water. The lower income populations are also less likely to purchase bottled water and have a greater prevalence of using infant formula than higher socio economic groups.

Similar geographic patterns of disease burden as reported in this study for respiratory disease, have also been reported for other inflammatory diseases.[69] Given the acknowledged role of inflammation in the pathology of cancer,[70],[71],[72] [73],[74] it is plausible that increased exposure to fluoride may also be a major contributory factor to the higher prevalence of cardiovascular disease, diabetes, rheumatoid arthritis, Alzheimer's autoimmune diseases and cancers incidence reported in fluoridated countries.[75]

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The Journal of the American Medical Association stated that *"science without criticality is unthinkable, for the only route to scientific objectivity is to question, not to 'accept'."* For too long the scientific community have accepted the endorsements of artificial fluoridation without the proper rigour of scientific assessment and those individuals, who in the interest of public health, that have questioned its efficiency, scientific merit or the thoroughness of scientific reviews have been ignored and criticised in the few countries that still support such a policy.

I also wish to acknowledge the many scientists, physician's, dental researchers and laypeople throughout the world that have raised scientific concern and awareness on the health

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EFFECT OF SODIUM FLUORIDE ON THE OXIDATIVE STRESS AND EXPRESSION OF BCL-2 AND BAX IN RAT MYOCARDIUM

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Abstracts

Introduction: Traditional fluorosis mainly refers to fluoride damage in hard tissue (teeth and bones). In recent years, soft tissue injury of fluoride has become a hot topic in researchers. Currently, cardiac effects of chronic fluorosis are almost confined to epidemiological investigation, and the conclusions are not consistent. In previous studies, we investigated the effects of fluorosis on the function of the heart through a heart perfusion model *in vitro* which indicated fluoride reduced the contractile function of the heart in response to ischemic stress.

Objectives: In this study, we mainly study the effect of sodium fluoride on the oxidative stress and the expression of Bcl-2 and Bax in rat myocardium.

Material and methods: Healthy adult Wistar rats were randomly divided into four groups. With one group as control, the other three groups were given sodium fluoride 50 ppm,100 ppm, and 200 ppm, respectively. After 12 and 16 weeks, the effect of different levels of fluoride on the oxidative stress and the expression of Bcl-2 and Bax in rat myocardium were observed through HE staining and immunohistochemical staining detection technology.

By measuring the protein content in myocardial tissue, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activity, and malondialdehyde (MDA) content, the lipid peroxidation injury of myocardial caused by fluorine in rats were studied.

Result: With increasing dosages of F, we found obvious pathological changes occurred in the myocardial tissue of rats. The amount of Bax expression showed a trend of rising overall in the myocardial cell, but with BCL - 2 it was the opposite. Results for peroxidation damage showed that excessive fluoride can inhibit the activity of CAT , GSH-Px and SOD in myocardial tissue which lead to the rise of MDA content.

Conclusion: When excessive fluorine is present in the body for a long time, it can cause myocardial cell apoptosis, and induce myocardial peroxidative damage, resulting in a change in the cardiac tissue structure. The results indicate that fluoride damages myocardial function to a certain extent, and its mechanism might be that lipid peroxidation was enhanced while antioxidant enzymes activity is suppressed because of fluorine accumulation which leaves body in the oxidative stress state, so as to induce myocardial peroxidative damage.

Keywords: Fluoride, Oxidative Stress, Rat Myocardium

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AN ENVIRONMENTAL STUDY: IS THERE CORRELATION BETWEEN FLUORIDE CONTENT OF DRINKING WATER AND HYPERTENSION?

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Abstract:
Besides damaging teeth and bones, the excessive intake of fluoride is known to cause a wide range of adverse health effects. When considering the adverse health effects of fluoride, the correlation of this important element with hypertension has still not been well studied. The aim of this study was to determine whether a relationship exists between the groundwater fluoride concentration and hypertension in people living in the villages in Bushehr Province in Iran. The data from 91 villages, with essentially the same socio-economic living standards and nutritional conditions, from 7 of the total of 9 districts in the Province, consisting of 160,150 habitants (including 80,661 males and 79,489 females) were analyzed in this study. The fluoride levels in the village drinking water measured by the SPADNS method ranged from 0.15 to 2.16 mg/L with a mean concentration of 1.03 mg/L, while the mean hypertension rate was 0.029, ranging from zero to 0.3. Over this concentration range, the results showed that there was no statistically significant correlation between the hypertension rate and the fluoride level of drinking water in the villages. Finally with regard to the little data on this topic, it seems more experimental and epidemiologic studies, especially at a higher fluoride content of drinking water, are necessary to have a better understanding about the relationship between the fluoride level of drinking water and hypertension.

Keywords: Bushehr; Fluoride in drinking water; Hypertension; Iran.

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Platform Session 3

Fluoride and the eye

FLUORIDE INCREASES OXIDATIVE BURDEN IN CATARACTOGENESIS: AN *IN VITRO* AND *IN VIVO* STUDY

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Abstract:

High fluoride exposure has been established to increase the prevalence of several diseases but there has been very limited study of the association of fluoride (F) and cataractogenesis. In both Rajasthan, India, and worldwide, cataract is one of the major causes of visual impairment and leads eventually to blindness. We selected 155 patients with cataract from a high ground drinking water F region, F> 2.5 ppm, and compared them with age- and sex-matched controls from a lower ground drinking water F region, F<1.5 ppm. Blood and natural lenses were collected for serum F measurement and assessment of the oxidative stress markers of lipid peroxide levels (LPO), protein carbonylation (PC), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) content. We observed a significant increase of LPO and PC in the subjects, as compared with the controls, in both the serum and the lenses ($p<0.001$). On the other hand, the antioxidant enzymes (SOD, CAT) and GSH were found to be markedly decreased in the subjects ($p<0.01$). No difference in vitamin A levels was present ($p>0.05$). On the basis of these results, it may be safely concluded that fluoride ingestion in high F endemic regions may directly influence cataractogenesis by increasing the oxidative burden.

Keywords: Cataract; Cataractogenesis; Fluoride and cataractogenesis; Oxidative stress; Oxidative stress and cataractogenesis; Rajasthan, India.

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IN-VITRO STUDY OF FLUORIDE-INDUCED BIOCHEMICAL CHANGES IN GOAT EYE LENS

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Abstract:

In the present study, we attempted to find out what biochemical changes fluoride (F) could cause in the lens of the goat eye. Twenty-four goat eye lenses were divided into four groups (n=6 per group). Three experimental groups were incubated with three different concentrations of F (50, 100 and 200 ppm) for 24 hours at room temperature and one group served as a control group. After incubation, the lenses were homogenised to estimate lipid peroxide level (LPO), protein carbonyl content (PC), total protein (TP), GSH and antioxidant enzymes such as SOD and CAT. We observed that LPO and PC gradually increased significantly ($p<0.001$) with the increased fluoride concentrations while there were significant decreases in the levels of reduced glutathione (GSH) and the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT). On the basis of these results, we concluded that the excessive consumption of F may lead to an increased oxidative burden resulting in lens opacification and the progression, at times, of this to the development of a cataract.

Key words: Antioxidant enzymes; Catalase; Fluoride effect on lens biochemistry; Fluoride-induced biochemical changes; Goat lens; GSH; Lens; Lipid peroxidation in the lens; Oxidative stress on lens; Protein carbonyl content; Superoxide dismutase.

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FLUORIDE AFFECTS QUALITY OF VISION: AN OBSERVATIONAL STUDY

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Abstract:

Fluoride (F) is abundantly distributed in many states of India and worldwide. A number of adverse effects have been reported from exposure of high levels of F in drinking water (>2.5 ppm). The effects of F on the eye are still being studied. The aim of the present study was to investigate the quality of vision, by carrying out a set of examinations, in residents in endemic fluorosis areas and to compare this to persons living in nonendemic fluorosis areas (F in drinking water<1.5 ppm). In this preliminary research, we recruited 175 subjects from rural areas with endemic fluorosis in Rajasthan, India, and age- and sex-matched controls from nonendemic areas. F was estimated in their serum and in their source of ground water. The subjects were given a routine ophthalmologic examination including examining vision; looking for conjunctival changes, lenticular changes, and retinal changes; corneal examination; testing for tear film abnormalities by Schirmer's test; and measuring the refractive status of the eyes. On the basis of results, which were found to be statistically significant ($p<0.001$), it was concluded that the prevalence of reduced vision quality and the associated ocular changes were more pronounced in the endemic areas as compared to the control nonendemic areas. Further study is warranted to better understand the ophthalmic toxicity of F.

Key words: Fluoride effects on the eye; Rajasthan, India; Retinal changes with fluorosis; Vision in fluorosis.

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Platform Session 4

Fluoride and the reproductive system

Jundong Wang

A RETROSPECTIVE LOOK AT THREE DECADES OF RESEARCH ON FLUORIDE TOXICITY

Abstract

1. A brief history of geological areas with high fluoride levels in China

China has geological areas with high fluoride levels and endemic fluorosis has accompanied the development there of human civilization. A 100,000-year-old fluorosed tooth fossil of homo sapiens was found at Dingcun of South Shanxi province. The 3,000-year-old fluorosed teeth of a warrior were discovered by archaeologists at Anyang city of Henan Province, where China's most significant archaeological findings of oracle bones have also been found. These 16th to 11th century B.C. bones or tortoise shell have inscriptions on them dating from the Shang Dynasty of slave society. Although finding archaeological evidence of the problem of endemic fluorosis has not been easy, solving it is much more difficult. Despite efforts being made for half a century to prevent fluorosis, their effectiveness has been limited. At present, endemic fluorosis is still present in 71,000 rural villages, with 21 million persons having dental fluorosis and one million skeletal fluorosis. Further attention will be needed for a long time to control and prevent endemic fluorosis.

2. Industrial fluoride pollution and animal fluorosis in China

During the last century some areas of China have been severely affected by industrial fluoride pollution resulting in fluorosis in animals involving impaired development. In order for herdsmen to be able to benefit economically from breeding animals, an effective and practical treatment of animal fluorosis was required. Dental fluorosis discolours the teeth but this is of secondary importance to the primary function of teeth for grazing animals of being able to eat grass for their nutrition. Our study demonstrated that a very significant improvement in animal tooth quality could be made by providing nutritional supplementation to the animals with micro-nutrients during the dry season. Even though it was not possible to stop the intake of fluoride by the animals, with feed supplements, the life expectancy of the animals could be extended to 4–5 years with improved health, productivity and fertility. For the herdsmen, there were significantly increased economic results from the healthier offspring and increased production of meat, down, and skin.

3. The influence of fluoride on brain and IQ

Our understanding and recognition of fluoride neurotoxicity have developed over a long time. During the 1990s, some investigators in China found that fluoride reduced the IQ in children by 8–10%. From 2000 onwards, more and more reports were published of investigations and experiments involving fluoride and IQ. We carried out two groups of experiments on the brains of rats. One studied the effect of high fluoride (F) and low iodine (I), and the other the effect of high F and high lead (Pb). The results, from behavioral data, transmission electron microscopy (TEM), DNA damage, cell apoptosis, gene (bcl-2, c-fos) expression, reverse transcription polymerase chain reaction (RT-PCR), qualitative and quantitative functional protein expression, and proteomic analysis of total brain and hippocampus, all provided strong evidence of brain damage from the combinations of high F and low I, and of F and Pb.

4. The influence of fluoride on male reproduction

Reproductive health is basic to the sustaining of life for both humans and animals. Experimental studies on animals in our laboratories showed fluoride had multiple negative effects, involving the morphology and function of the testis, epididymis, vas deferens, and sperm. After examining sperm hyperactivation, sperm chemotaxis, some target genes, microRNA (miRNA) microarray, proteomic analysis, testis RNA-Seq procedure (Whole Transcriptome Shotgun Sequencing, WTSS), and signal pathways, we found that fluoride disrupted the normal reproductive system through a variety of mechanisms.

Key words: Animal fluorosis; Fluoride neurotoxicity; Fluoride reproductive toxicity; History of endemic fluorosis; Retrospective look at fluoride research.

Full Paper

1. A brief history of geological areas with high fluoride levels in China

China has geological areas with high fluoride levels and endemic fluorosis has accompanied the development there of human civilization. A 100,000-year-old fluorosed tooth fossil of homo sapiens was found at Dingcun of South Shanxi province. The 3,000-year-old fluorosed teeth of a warrior were discovered by archaeologists at Anyang city of Henan Province, where China's most significant archaeological findings of oracle bones have also been found. These 16th to 11th century B.C. bones or tortoise shell have inscriptions on them dating from the Shang Dynasty of slave society.

Although finding archaeological evidence of the problem of endemic fluorosis has not been easy, solving it is much more difficult. In theory, stopping the drinking of high F water will naturally lead to fluorosis stopping. However, while this is easy to say, putting it into practice is not. Remedial measures to reduce fluoride exposure cannot be applied over all of the high fluoride areas because of vast area involved and some techniques are effective for only a limited time.

Despite efforts being made for half a century to prevent fluorosis, their effectiveness has been limited. At present, endemic fluorosis is still present in 71,000 rural villages, with 21 million persons having dental fluorosis and one million skeletal fluorosis. For example, Shanxi province has 119 counties and in 66 of these there is still endemic fluorosis resulting from drinking water with high fluoride levels. A quarter of the rural population, 23 million, still drink water with high fluoride levels. South Shanxi, including Linfen and Yuncheng, has a serious problem with endemic fluorosis.

Figure1. Fluoride content in groundwater of China. The rectangle is Shanxi Province.

According to my experience and observations, fluorosis is, in general, a disease of the poor in China. In my view, improving nutrition is the best way to alleviate fluorosis in poor areas. For children, during their development, there is a need for proper nutrition, including an adequate intake of protein and calcium, in order to ensure healthy growth. At present many migrant workers are moving from country areas with high fluoride levels to large cities with lower fluoride levels for employment and this will help to prevent or alleviate fluorosis.
Further attention will be needed for a long time to control and prevent endemic fluorosis.

2. Industrial F pollution and animal fluorosis in China

During the years 1975–1985 animal fluorosis, was present in many provinces due to fluoride emissions from smelting factories. The most serious pollution was from the Baotou Steel and Iron Group in Inner Mongolia. When the Chinese government became aware of the problem, fluoride emissions were prohibited from many small- to mid-sized factories and animal fluorosis from industrial fluoride pollution disappeared in most provinces. However, because the Baotou Steel and Iron Group had an important role in national steel production, closing it is not possible and the opportunities for reducing pollution by technical improvements were limited.
The Baotou Steel and Iron Group factories are situated in a pasturing area of Inner Mongolia where there are 600,000 animals including sheep, goats, cattle, horses, and camels. A conflict exists between the need for iron and steel production by industry with accompanying fluo-

ride pollution and the need to minimize pollution to ensure healthy animals for animal-based production. The heavy industrial pollution from fluoride led to the disappearance from the adjacent area of large animals, such as cattle and horses, because no economic benefit was possible with unhealthy animals. Only small animals, such as goats and sheep, were kept, but even these animals lived for only two or three years due to the excessive wear on some teeth that were weakened by dental fluorosis. The challenge for the researchers was to find measures to reduce or alleviate the fluoride toxicity so that the herdsmen could continue to support themselves by breeding animals that were healthy and productive with normal fertility

Before 1987, researchers investigated alleviating the effects of fluoride pollution by the use of various elements, including Ca, Al, Cu, and Se, but although some improvement was seen at a biochemical level no clinical effects were apparent. Only moving animals from fluoride-polluted areas to non-fluoride-polluted areas was helpful but this was not a practical solution because of the issues of ownership of pasture land.

In order for the herdsmen to get economic benefits from animal breeding, some effective and practical measures had to be found. From our observations and investigations in 1987–1991, we discovered that the industrial animal fluorosis in this area was very different from that of the usual endemic fluorosis. In contrast to endemic fluorosis caused by a constantly raised water fluoride level, the grass fluoride levels in industrial animal fluorosis varied greatly from a high of up to 70 ppm in dry seasons to a low of 10ppm in the rainy seasons. The bone fluoride levels varied from 7,000 ppm in the dry seasons to 4,000 ppm in rainy seasons. The length of the teeth also varied with some teeth being shorter while others were longer.

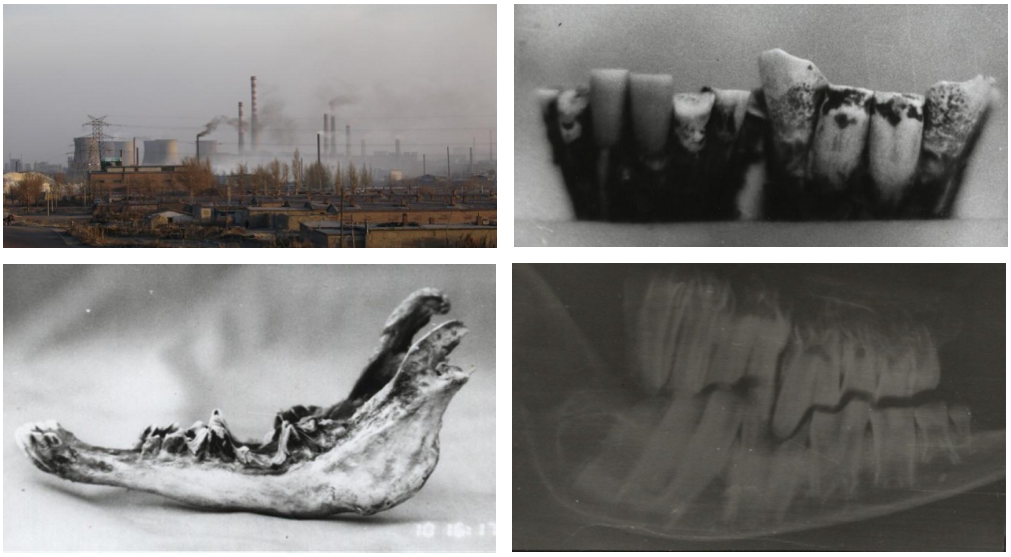


Figure 2. Baotou Steel and Iron Group factories and fluorosed animal teeth

We determined that the nutritional value of the grass was low in the dry seasons and high in

the rainy seasons. Green grass has 13%–17% of protein, a digestibility level of 65%, and 10 MJ/Kg of metabolic energy, while dry grass has 5% of protein, a digestibility level of 33%, and 4.5 MJ/Kg of metabolic energy.

We observed that the cause of the animals not eating grass and finally dying of hunger was the excessive wear on some teeth which developed in the dry seasons when the grass was high in fluoride and low in nutritive value. Our preliminary hypothesis was that the combination of the high fluoride content and the poor nutritional value of the dry grass was the cause of the excessive tooth wear.

Although we were unable to stop the fluoride pollution, we considered whether or not the tooth quality could be improved to some extent by supplementing the animal nutrition in the dry seasons with some micro-nutrients. We considered that the protein and energy intake would be more important for body and tooth development than other micro-nutrients. Our experiments with supplementation showed that additional protein and energy resulted in significantly improved tooth quality, although the dicolouration from the dental fluorosis still persisted. Furthermore, animals were able to live for 4–5 years instead of 2–3 years and experienced improved health, productivity and fertility. For the herdsmen, there were significantly increased economic results from the healthier offspring and increased production of meat, down, and skin.

In conclusion, we found that although feed supplementation in the dry seasons did not stop the intake of fluoride, it was an effective measure to alleviate fluoride toxicity, and it enabled the herdsmen have a living based on raising animals. .

3. Influence of fluoride on brain

The Intelligence Quotient (IQ) is a basic parameter of health for humans and a reduction in IQ will decrease a person's capacity to live and work. We know that many environmental factors such as low iodine and high lead levels can severely affect IQ. Whether fluoride can affect IQ is an important question. In the 1990's when the effect of fluoride on teeth and bones was being studied, what did researchers think about the influence of fluoride on brain and IQ?

In 1995, some investigators found the IQ in children was reduced by 8–10% in the Chinese provinces of Guizhou (Li XS,1995[1]) and Shanxi (Zhao LB,1996[2]). This was also the year that Mullenix also published her paper on the effect of fluoride on rat behavior.[3] Later, we found that she obtained her first data to show that fluoride affected the brain in 1989.[4] Her important findings have been cited over 250 times. In 2000, Lu Y reported that the IQ of children in the endemic fluorosis area of Tianjin in China was reduced by 8–10%.[5] On reviewing the literature, we found that Li FF (1991) found a much greater reduction of IQ of 19–25% in Xinjiang in China which was affected by high fluoride and low iodine.[6] On basis of these reports, Spittle (2000) published an editorial, "Fluoride and IQ" in *Fluoride* in which he noted that when observational data can be replicated consistently, they became relatively secure and

less likely to be in error.[7] He considered that the relationship linking fluoride to diminished intelligence was becoming more firmly established and that further research on the neurotoxic effects of the interaction between fluoride and iodine would be worthwhile.

We carried out two groups of experiments on the brains of rats. In one we studied the effect of high fluoride (F) and low iodine (I), and in the other the effect of high F and high lead (Pb). The results, from behavioral data, transmission electron microscopy (TEM), DNA damage, cell apoptosis, gene (bcl-2, c-fos) expression, reverse transcription polymerase chain reaction (RT-PCR), qualitative and quantitative functional protein expression, and proteomic analysis of total brain and hippocampus, all provided strong evidence of brain damage from the combinations of high F and low I, and of F and Pb.

From 2002 to 2006, we studied the effects of high fluoride and low on the brains of rats with applied behavioral observations for learning and memory, histopathology by TEM, biochemical determinations including for oxidative damage and ChE activity, DNA damage, cell apoptosis, RT-PCR, gene (bcl-2,c-fos) expression, and the brain protein content in offspring rats, especially by using proteomic analysis of total brain with two-dimensional electrophoresis for finding different proteins . The results showed that the damage associated with low iodine was much greater than with high fluoride, and that the most severe toxicity occurred with the combination of high fluoride and low iodine. Our conclusion, from the variety of investigations was that there was strong evidence that the combination of high fluoride and low iodine could cause brain damage.

As is well known, lead (Pb) is another element that can severely affect brain development and IQ. The 2003 paper by Canfield et al. in the NEJM indicated that Pb was able to reduce IQ even at blood Pb levels less than 0.1 mg/L (10µg/dL).[8] Rogan et al., in a paper "Exposure to lead in children—how low is low enough?", showed that blood Pb level thought to be safe in the USA progressively decreased from 0.60 mg/L in 1970 to 0.40, 0.30, 0.20, 0.10, and finally, in 1999, to 0.020mg/L.[9] In 2003, when Xiang QY's investigation showed that the IQ in children was reduced by 8%, from a mean IQ of 100.41 to a mean IQ of 92.02, in Jiangsu province, where low iodine was not present,[10] Professor Burgstahler queried, in an editorial in *Fluoride*, "Influence of fluoride and lead on children's IQ: U.S. tolerance standards in question," whether or not high lead levels might be affecting the results.[11]

We studied the influence of fluoride and lead on the brain and IQ with systematic investigations of the alterations in nervous system of adolescent and adult rats with exposure to fluoride. In particular, we used a variety of behavioral observations, qualitative and quantitative functional protein expression, and proteomic analysis of the total brain and the hippocampus. We found that both fluoride and lead adversely affected brain development.

I believe that, in conjunction with the efforts of our colleagues, future research on fluoride and the brain will continue to make important discoveries.

4. The influence of fluoride on male reproduction

Reproductive health is basic to the sustaining of life for both humans and animals. Without healthy male reproductive functioning, life in humans and in the animals important to farmers and herdsmen is not possible. In the past 20 years, the infertility rate in China has increased from 3% to 12% with 40 million patients being affected. Male reproductive function is adversely affected by many environment factors.

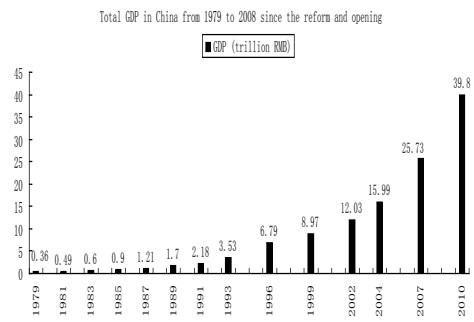
Compared to the epidemiological investigations of fluoride-induced neurotoxicity, there are few human studies on fluoride and fertility. After Freni reported in 1994 that drinking water with high fluoride levels decreased birth rates,[12] further research has shown that in high fluoride areas there are changes in hormone levels in humans, particularly decreased testosterone. In the period 1990–2006, more than 50 publications have focused on the reproductive effects of fluoride. In 2009, a review of the effects of fluoride toxicity on the male reproductive system was published with 135 references.[13] Editorials in journal *Fluoride* in 2008 and 2009 also emphasized the importance of research on the reproductive system toxicity of fluoride.[14,15]

Experimental studies on animals, in both our laboratories and those of others, showed fluoride had multiple negative effects, involving the morphology and function of the testis, epididymis, vas deferens, and sperm.

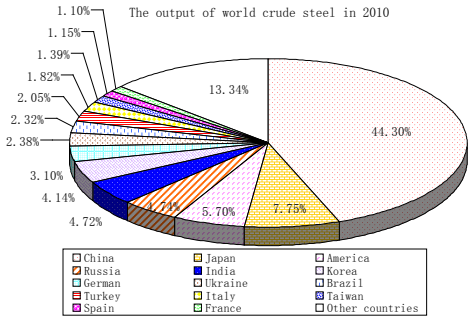
In order to deepen our understanding of the mechanisms by which fluoride affected reproduction, we chose and analyzed dependent target genes and proteins by applying omics technology. Proteomic analysis of testicular proteins of mice exposed to different doses of fluoride showed some important findings. After examining sperm hyperactivation, sperm chemotaxis, some target genes, microRNA (miRNA) microarray, proteomic analysis, testis RNA-Seq procedure (Whole Transcriptome Shotgun Sequencing, WTSS), and signal pathways, we found that fluoride disrupted the normal reproductive system through a variety of mechanisms.

5. Environmental pollution from fluoride

It is well known that China's economic development has been very fast, with its gross domestic product (GDP) being ranked second in the world and having a value of half that of the USA which is ranked first. In 2012, steel and cement production in China accounted for 44% and 60% respectively of the world production. In the past decade, this rapid economic development has resulted in severe environmental problems. Shanxi Province leads China is coal production and if a train could be made that contained all the coal produced in China in a year, its length would be such as to be able to encircle the earth three times. As the GDP in China has increased, the air and water have become dirtier and dirtier.



GDP sum is second in the world



2010: World steel production

Figure 3. The environmental pollution that has accompanied the increase in China's GDP.

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THE ANTAGONIST EFFECTS OF FLUORIDE ON THE METAL IONS, Fe, Se, Cu AND Zn, IN SEMINAL PLASMA AND ITS INFLUENCE ON SPERMATOGENESIS

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Abstract:

Fluorosis has become a worldwide endemic problem, including in India, with effects on various organ systems, including spermatogenesis. Excessive fluoride has been associated with reduced sperm quality but the mechanism of action for this is still unknown. Iron (Fe), selenium (Se), copper (Cu), and zinc (Zn) are trace elements essential for normal spermatogenesis of mammals and play a critical role with antioxidant defence system enzymes. The present study was designed to investigate the metal antagonistic effects of fluoride (F) and to correlate this with serum F levels in fluorotic patients. Fifty-five fluorotic patients, aged 25–4, were recruited. The controls were selected from the Jaipur district, Rajasthan, India, where the fluoride content in the ground water was <1.5 ppm. Fasting blood samples was collected for the estimation of serum F levels and semen samples were also collected for semen profiles and measurement of the concentrations of Fe, Cu, Zn and Se. The results demonstrated that sperm count, motility, viability and liquefaction time were altered in the fluorotic patients compared to the controls ($p<0.05$). The concentrations of Cu and Fe were higher in the fluorotic patients while the levels of Se and Zn were reduced ($p<0.01$). On the basis of these results, we concluded that Cu and Fe are key mediators of the oxidative stress and impairment of spermatogenesis caused by fluoride. Se and Zn are cofactors for the antioxidant enzymes glutathione peroxidase and superoxide dismutase respectively. Significant correlations were found between the concentration of F in the serum and the levels of trace metals in the seminal plasma. The results indicate that specific nutritional supplements may be of benefit for fluoride-effected populations. However, further in-depth studies are required to more fully understand the pathophysiology of fluoride-induced reproductive toxicity.

Key Words: Copper; Fluoride effects on metal ions; Fluoride effects on spermatogenesis; Iron, Metals, Selenium; Semen; Seminal plasma; Zinc.

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ENDEMIC FLUOROSIS ELEVATION OF THE OXIDATIVE BURDEN IN PREGNANCY: A CORD BLOOD STUDY

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Abstract:

It has been reported that the mitochondrial rich placenta may be an oxidative burden in pregnant women and that fluoride passing from the mother to the placenta may interfere with foetal development. The aim of the present study was to evaluate the effect of fluoride-dependent oxidative stress in pregnant women. We recruited 53 pregnant women subjects who were residing in a high fluoride region of the Rajasthan, India and control women from a non-endemic area of the Rajasthan. The cord blood was collected for measurement of oxidative stress markers i.e., lipid peroxide level, superoxide dismutase, catalase, and reduced glutathione. We observed significantly increased lipid peroxide levels and reduced antioxidant status in the subjects when compared with the controls ($p<0.001$). On the basis of these results, we concluded that excessive fluoride potentiates oxidative stress in pregnancy and suggest that antioxidant supplementation to the mother during pregnancy may be of value. Further study, however, is required of this area.

Key words: Fluoride, cord blood, pregnancy, oxidative stress

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Platform Session 5

Fluoride, bones and teeth

Bruce James Spittle

DENTAL CARIES IN NEW ZEALAND IN 2011: THE EFFECTS OF SOCIOECONOMIC STATUS AND WATER FLUORIDATION

Abstract

Objective: To examine the influence on dental decay in five-year-old and Year 8 (12-year-old) children of water fluoridation and socioeconomic status.

Method: The 2011 Ministry of Health data on dental health status for five-year-old and Year 8 children were used to provide information on dental decay in fluoridated and non-fluoridated areas for the 20 district health boards. An index for socioeconomic status was created using the Ministry of Health deprivation 2013/14 quintiles. Statistical analysis was by two-way ANOVA and linear regression.

Results: No significant differences in percentage caries-free or mean dmft were present in either five-year-old or Year 8 children related to fluoridation status. In contrast, although dental decay, percentage caries-free or mean dmft, was not significantly affected by the DHB region in the Year 8 children, a significant effect, $p<0.05$ for percentage caries free and $p<0.01$ for mean dmft, was present in the five-year-old children. Linear regression analysis showed that dental decay, both percentage caries-free and mean dmft, in five-year-olds, in total for all of New Zealand, and for the fluoridated- and the non-fluoridated areas, was significantly related, to lower socioeconomic status, (% caries-free: $p<0.0001$, $p=0.0030$ and $p=0.0002$ respectively: mean dmft: $p<0.0001$, $p=0.0006$ and $p=0.0001$ respectively). No significant relationships were present for Year 8 children.

Conclusions: The 2011 oral health data indicate that in 2011 dental decay in five-year-old and Year 8 (12-year-old) New Zealand children is not significantly influenced by water fluoridation but that significant increases in dental decay in five-year-olds, but not Year 8 children are associated with lower socioeconomic status. Although other non-ecologic studies in the literature indicate that water fluoridation reduces dental decay in five-year-olds, particularly in low- and mid-socioeconomic groups, there is evidence that this is mediated by impairing thyroid hormone levels with an accompanying risk of systemic toxicity including impairment of brain development.

Keywords: Dental caries and socioeconomic status, Dental caries and water fluoridation, Dental caries in New Zealand.

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Introduction

Dental caries remains a prevalent chronic disease in New Zealand affecting both children and adults (Grant et al., 2013) and is considered to be influenced by both socioeconomic status and water fluoridation (Kilpatrick et al., 2008; Ministry of Health, 2010).

Although supported by most New Zealand general dental practitioners (Grant et al., 2013), water fluoridation has remained a contentious issue since it commenced on 25 January 1945 in Grand Rapids, Michigan, USA. On 5 June 2013, following a four-day tribunal, the Hamilton City Council voted 7 to 1 to discontinue water fluoridation which had started in the city to prevent dental caries in 1966. Shortly afterwards, on 12 June 2013, the Office of the Prime Minister's Science Advisory Committee issued a statement noting that both the science of fluoride in water and the genuine concerns that had been raised regarding risks of bone disease, thyroid disease, brain disease, and cancer, had been settled (Office of the Prime Minister's Science Advisory Committee, 2012). Examples of these concerns are papers by Carton on bone, thyroid, and brain disease, by Choi et al., on brain disease and by Bassin et al. on cancer (Carton, 2006; Choi et al., 2012; Bassin et al., 2006). The ethical basis of fluoridation has also been challenged recently (Awofeso, 2012).

Similarly, in October 2012, a statement by Professor Sir David Skegg, President of the Royal Society of New Zealand noted that extensive research over many decades indicated that fluoridation was a safe and effective measure for reducing the incidence of dental caries and that his only regret was that children in several areas of New Zealand were currently being deprived of this effective public health measure (Skegg, 2012).

The New Zealand College of Public Health Medicine also endorsed community water fluoridation as an important public health measure in the maintenance of oral health, the prevention of tooth decay and the reduction of health inequalities in a policy statement in June 2013 (New Zealand College of Public Health Medicine, 2013).

Cheng et al. noted that although the prevalence of caries varies between countries, levels everywhere have fallen greatly in the past three decades, regardless of the concentration of fluoride in water or the use of fluoridated salt (Cheng et al., 2007). They considered that this probably reflected the use of fluoridated toothpastes and other factors, including perhaps aspects of nutrition.

Falling dental decay rates have also been seen in New Zealand and in this fluid situation it was considered appropriate to examine the statistical significance of the 2011 Ministry of Health data on dental health status of five-year-old children and Year 8 (12-year-old) children and to relate the dental decay information from the 20 district health boards to an index of socioeconomic status for each board (Brown, 1988).

In studying the effect of socioeconomic status on dental caries, the sample size in several New Zealand studies was less than 1,500 and for an Australian study it was less than 7,500 (Evans et al., 1984; Fergusson and Horwood, 1986; Thomson et al., 2004a, 2004b; Treasure

and Dever, 1992, 1994; Lee and Dennison, 2004; Mackay and Thomson, 2005; Schluter et al., 2008; Kanagaratnam et al., 2009; Kamel et al., 2013, Slade et al, 1996). In the present study, the Ministry of Health data on dental health status of five-year-old children and Year 8 (12-year-old) children in 2011 were examined with respect to socioeconomic status because they offered a larger sample size with each group being above 44,500 in size.

The 2011 Ministry of Health data on dental health status of five-year-old children and Year 8 (12-year-old) children reported the New Zealand totals, for both the percentage of caries free teeth (% caries free) and the mean number of decayed, missing and filled teeth (dmft), for fluoridated and non-fluoridated areas but did not include any statistics to indicate whether the results for these areas were significantly different (Ministry of Health, 2012). The percentage of caries free five-year-old children in the fluoridated areas was 59.91% and 59.18% in the non-fluoridated areas. For Year 8 children, the % caries free figures were 55.17% and 51.79% respectively. For five-year-old children in fluoridated areas, the mean dmft was 1.77 and for the non-fluoridated areas it was 1.90. For Year 8 children, the mean dmft figures were 1.14 and 1.37 respectively. In the present study, the statistical significance of these results is examined.

Methods

The data for dental caries (% caries free and mean dfmt) for five-year-old children and Year 8 children in 2011, for the twenty district health board (DHB) regions, were taken from the Ministry of Health website (Ministry of Health, 2012a).

The data for socioeconomic status for the twenty DHB regions were the deprivation, 2013/14, quintiles taken from the Ministry of Health website (Ministry of Health, 2012b). For a socioeconomic status index (SESI), the percentages for quintiles 4 and 5 (most deprived) were added and the total divided by the percentages for quintiles 1 (least deprived) and 2. A higher score indicates more deprivation or lower socioeconomic status.

$$\text{SESI} = \frac{\%\text{ for quintile 4} + \%\text{ for quintile 5}}{\%\text{ for quintile 1} + \%\text{ for quintile 2}}$$

The statistical analysis was done using GraphPad Prism 6™ using two-way ANOVA and linear regression (GraphPad Software, 2013).

Results

The SESIs for the 20 district health board regions are shown in Table 1.

Table 1. Deprivation, 2013/14 quintiles (quintile 1: least deprived; quintile 5: most deprived) and socioeconomic status indexes (SESIs) for the district health board (DHB) regions

District health board region	Deprivation quintile					SESI
(n=total population, children and adults, in the region)	1	2	3	4	5	
Auckland DHB (n=469,400)	21	21	19	20	19	0.929
Bay of Plenty DHB (n=214,910)	14	19	22	21	24	1.364
Canterbury DHB (n=509,860)	27	23	21	17	12	0.580
Capital & Coast DHB (n=299,720)	31	22	19	14	14	0.528
Counties Manukau DHB (n=516,050)	19	16	13	17	35	1.486
Hawke's Bay DHB (n=156,490)	16	16	21	21	26	1.469
Hutt Valley DHB (n=145,215)	25	15	18	20	22	1.050
Lakes DHB (n=103,170)	18	17	14	21	30	1.457
Midcentral DHB (n=170,200)	16	16	22	24	22	1.438
Nelson-Marlborough DHB (n=141,933)	19	25	24	25	7	0.727
Northland DHB (n=159,759)	9	14	21	24	32	2.435
South Canterbury DHB (n=56,695)	18	28	24	21	9	0.652
Southern DHB (n=309,028)	24	23	21	19	13	0.681
Tairawhiti DHB (n=46,753)	10	12	13	20	45	2.955
Taranaki DHB (N=110,258)	12	21	26	24	17	1.242
Waikato DHB (n=373,220)	15	19	20	22	24	1.353
Wairarapa DHB (n=40,735)	20	18	18	29	15	1.158
Waitemata DHB (n=562,970)	27	25	22	18	8	0.500
West Coast DHB (n=33,055)	9	20	20	35	16	1.759
Whanganui DHB (n=62,630)	10	15	20	21	34	2.200
Total for all DHB regions (n=4,482,051)						

The data on dental caries (% caries free and mean dmft) for five-year-olds and Year 8 children are shown in Tables 2 and 3.

Table 2. Dental caries (% caries free and mean dmft) for five-year-olds in total and for fluoridated (F) and non-fluoridated (non-F) areas						
District health board (DHB) region (n=total 5-yr-olds n; n in F; n in non-F)	% caries free			Mean dmft		
	Total	F	Non-F	Total	F	Non-F
Auckland DHB (n=4,114; 3,874; 240)	61.62	62.00	55.42	1.70	1.67	2.15
Bay of Plenty DHB (n=1,659; 79; 1,580)	50.03	65.82	49.24	2.39	1.70	2.42
Canterbury DHB (n=4,810; 65; 4,745)	63.66	53.85	63.79	1.49	1.92	1.48
Capital & Coast DHB (n=2,649; 2,633; 16)	74.48	74.48	75.00	0.98	0.98	0.69
Counties Manukau DHB (n=5,978; 5,525; 453)	47.59	46.70	58.50	2.49	2.54	1.83
Hawke's Bay DHB (n=1,714; 840; 874)	53.97	54.88	53.09	1.80	1.77	1.82
Hutt Valley DHB (n=1,420; 1,348; 72)	60.35	60.98	48.61	1.58	1.55	2.14
Lakes DHB (n=1,176; 297; 879)	58.67	47.47	62.46	2.68	1.84	2.97
Midcentral DHB (n=1,796; 1,087; 709)	59.91	62.83	55.43	1.58	1.33	1.97
Nelson-Marlborough DHB (n=1,435; 0; 1,435)	67.32	0	67.32	1.30	0	1.30
Northland DHB (n=1,112; 0; 1,112)	35.25	0	35.25	3.55	0	3.55
South Canterbury DHB (n=618; 0; 618)	58.09	0	58.09	1.68	0	1.68
Southern DHB (n=2,960; 1,443. 1,517)	70.30	71.17	69.48	1.06	1.01	1.11
Tairawhiti DHB (n=734; 539; 195)	41.55	42.67	38.46	3.11	3.11	3.12
Taranaki DHB (n=1,499; 1,050; 449)	56.17	57.90	52.12	1.96	1.91	2.08
Waikato DHB (n=3,689; 1,830; 1,859)	60.88	57.60	64.12	2.20	2.29	2.11
Wairarapa DHB (n=431; 215; 216)	67.05	64.65	69.44	1.30	1.37	1.23
Waitemata DHB (n=5,524; 5,024; 500)	65.06	65.51	60.60	1.44	1.41	1.68
West Coast DHB (n=488; 0; 488)	61.07	0	61.07	1.87	0	1.87
Whanganui DHB (n=847; 0; 847)	53.01	0	53.01	1.96	0	1.96
Total for all DHBs (n=44,653; 25,849; 18,804)	59.60	59.91	59.18	1.82	1.77	1.90
% increase in F compared to non-F for all DHBs		1.23				
% increase in non-F compared to F for all DHBs						7.34

Table 3. Dental caries (% caries free and mean dmft) for Year 8 (12-year-olds) in total and for fluoridated (F) and non-fluoridated (non-F) areas						
District health board region (n=total Year 8 n; n in F; n in non-F)	% caries free			Mean dmft		
	Total	F	Non-F	Total	F	Non-F
Auckland DHB (n=4,466; 4,339; 127)	58.51	58.77	49.61	1.03	1.02	1.44
Bay of Plenty DHB (n=1,836; 103; 1,733)	49.84	61.17	49.16	1.67	1.69	1.67
Canterbury DHB (n=4,832; 84; 4,748)	56.54	39.29	56.84	1.00	1.58	0.99
Capital & Coast DHB (n=2,563; 2,512; 51)	66.37	66.56	56.86	0.71	0.70	0.84
Counties Manukau DHB (n=5,182; 4,735; 447)	47.39	46.99	51.68	1.44	1.47	1.13
Hawke's Bay DHB (n=1,277; 699; 578)	53.95	57.65	49.48	1.25	1.09	1.44
Hutt Valley DHB (n=1,538; 1,488; 50)	59.10	59.48	48.00	0.90	0.89	1.22
Lakes DHB (n=1,318; 309; 1,009)	38.16	52.10	33.89	2.03	1.31	2.25
Midcentral DHB (n=2,122; 1,276; 846)	48.26	45.06	53.07	1.50	1.56	1.41
Nelson-Marlborough DHB (n=1,682; 0; 1,682)	55.71	0	55.71	1.04	0	1.04
Northland DHB (n=1,068; 0; 1,068)	50.19	0	50.19	2.30	0	2.30
South Canterbury DHB (n=664; 0; 664)	51.36	0	51.36	1.29	0	1.29
Southern DHB (n=3,148; 1,743; 1,405)	48.60	49.80	47.12	1.26	1.19	1.34
Tairawhiti DHB (n=688; 565; 123)	54.94	55.93	50.41	1.06	1.03	1.24
Taranaki DHB (n=1,566; 1,147; 419)	51.72	52.75	48.93	1.11	1.12	1.09
Waikato DHB (n=4,418; 2,283; 2,135)	55.14	56.72	53.44	1.50	1.36	1.64
Wairarapa DHB (n=411; 180; 231)	49.64	52.22	47.62	1.24	1.26	1.22
Waitemata DHB (n=4,679; 4,095; 584)	57.30	57.53	55.65	0.99	0.99	1.01
West Coast DHB (n=395; 0; 395)	47.59	0	47.59	1.39	0	1.39
Whanganui DHB (n=806; 0; 806)	50.87	0	50.87	1.31	0	1.31
Total for all DHBs (n=44,659; 25,558; 19,101)	53.72	55.17	51.79	1.24	1.14	1.37
% increase in F compared to non-F for all DHBs		6.53				
% increase in non-F compared to F for all DHBs						20.18

The results of the two-way analysis of variance (two-way ANOVA) of the effect on dental caries (% caries free and mean dmft) of DHB region and fluoridation status with residence in a fluoridated (F) or a non-fluoridated (non-F) area, in five-year-olds and Year 8 (12-year-olds) are shown in Table 4.

Table 4. Effect on dental caries (% caries free and mean dmft) of district health board (DHB) region and fluoridation status, with residence in a fluoridated (F) or a non-fluoridated (non-F) areas, in five-year-olds and Year 8 (12-year-olds)

Factor	5-year-olds		Year 8	
	% caries free p value (% of total variation) [F ratio]	mean dmft p value (% of total variation) [F ratio]	% caries free p value (% of total variation) [F ratio]	mean dmft p value (% of total variation) [F ratio]
DHB region	p=0.0171 (76.38%) [F=3.265]	p=0.0020 (82.26%) [F=5.169]	p=0.4882 (45.39%) [F=1.016]	p=0.0823 (66.03%) [F=2.149]
Fluoridation status	p=0.7189 (0.2254%) [F=0.1349]	p=0.2257 (1.826%) [F=1.606]	p=0.0992 (9.949%) [F=3.118]	p=0.2440 (3.246%) [F=1.479]
p-values calculated using two-way ANOVA.				

The results of the linear regression analysis of the effect on dental caries (% caries free and mean dmft) of the SESI in five-year-olds and Year 8 (12-year-olds) in total, in fluoridated (F) areas and in non-fluoridated (non-F) areas are shown in Table 5.

Table 5. Effect on dental caries (% caries free and mean dmft) of the SESI in five-year-olds and Year 8 (12-year-olds) in total, in fluoridated (F) areas and in non-fluoridated (non-F) areas,

Factor	5-year-olds		Year 8	
	% caries free p value (F ratio) [nv=total number of values, nmv=number of missing values]	mean dmft p value (F ratio) [nv=total number of values, nmv=number of missing values]	% caries free p value (F ratio) [nv=total number of values, nmv=number of missing values]	mean dmft p value (F ratio) [nv=total number of values, nmv=number of missing values]
Total (F and non-F areas)	p<0.0001 (F=35.99) [nv=20, nmv=0]	p<0.0001 (F=33.87) [nv=20, nmv=0]	p=0.1341 (F=2.461) [nv=20, nmv=0]	p=0.0299 (F=5.561) [nv=20, nmv=0]
F areas	p=0.0030 (F=13.20) [nv=15, nmv=5]	p=0.0006 (F=20.35) [nv=15, nmv=5]	p=0.9812 (F=0.0005762) [nv=15, nmv=5]	p=0.6445 (F=0.2231) [nv=15, nmv=5]
Non-F areas	p=0.0002 (F=20.70) [nv=20, nmv=0]	p=0.0001 (F=23.09) [nv=20, nmv=0]	p=0.2371 (F=1.496) [nv=20, nmv=0]	p=0.0361 (F=5.127) [nv=20, nmv=0]
p-values calculated using linear regression.				

The linear regressions between socioeconomic status, measured by SESI, and dmft in all five-year-olds and all Year 8 (12-year-old) children are shown in Figures 1 and 2.

Figure 1. Linear regression between dmft in all 5-year-olds (F- and non-F areas) and SESI.
p<0.0001
F=33.87
number of values=20
number of missing values=0]

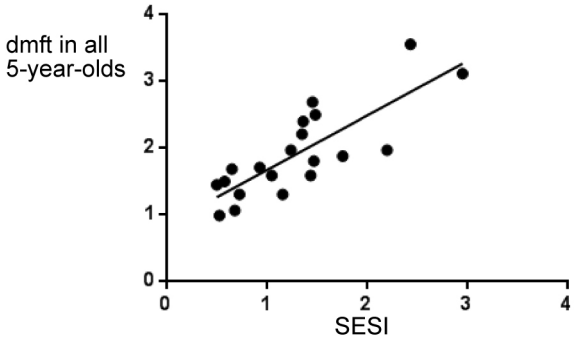
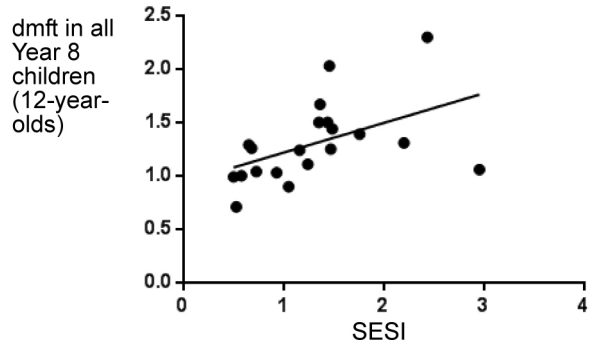


Figure 2. Linear regression between dmft in all Year 8 children (12-years olds in F- and non-F areas) and SESI.
p=0.0299
F=5.561
number of values=20
number of missing values=0



For Year 8 children in the higher- and lower-socioeconomic status quintiles (n=34,905), 30.13% (n=10,518) were in higher-socioeconomic status quintiles (1 and 2) in fluoridated areas, 26.52% (n=9,258) were in lower-socioeconomic status quintiles (4 and 5) in fluoridated areas, 21.66% (n=7,562) were in higher-socioeconomic status quintiles (1 and 2) in non-fluoridated areas, and 21.68% (n=7,567) were in lower-socioeconomic status quintiles (4 and 5) in non-fluoridated areas. Whereas the Year 8 children in the non-fluoridated areas were evenly distributed between the high- and low-socioeconomic status areas, for the children in the fluoridated areas 3.61% more were in a high- rather than a low- socioeconomic status area ($p<0.0001$ with testing by chi-squared with Yates' correction, $c^2 = 35.07$, degrees of freedom=1). The 3.61% difference in distribution for the children in the fluoridated areas corresponded to a difference in the dmft of approximately 0.05.

For the five-year-olds no significant difference in distribution between the fluoridated- and the non-fluoridated areas was present for those in the high- rather than a low- socioeconomic status areas ($p=0.9625$ with testing by chi-squared with Yates' correction, $c^2 = 0.002207$, degrees of freedom=1).. For five-year-old children in the higher- and lower-socioeconomic status

quintiles (n=35,496), 30.24% (n=10,734) were in higher-socioeconomic status quintiles (1 and 2) in fluoridated areas, 28.94% (n=10,273) were in lower-socioeconomic status quintiles (4 and 5) in fluoridated areas, 20.87% (n=7,408) were in higher-socioeconomic status quintiles (1 and 2) in non-fluoridated areas, and 19.95% (n=7,081) were in lower-socioeconomic status quintiles (4 and 5) in non-fluoridated areas.

Thus the two-way ANOVA of the 2011 Ministry of Health data on dental health status of five-year-old children and Year 8 (12-year-old) children indicated that there were no significant differences in percentage caries-free or mean dmft in either five-year-old ($p=0.7189$ and $p=0.2257$ respectively) or Year 8 (12-year-old) ($p=0.0992$ and $p=0.2440$ respectively) children related to fluoridation status (Table 4). In contrast, although dental decay, percentage caries-free or mean dmft, was not significantly affected by the DHB region in the Year 8 children ($p=0.4882$ and $p=0.0823$ respectively), a significant effect, $p<0.05$ for % caries free and $p<0.01$ for mean dmft, was present in the five-year-old children (Table 4). Linear regression analysis showed that dental decay, both percentage caries-free and mean dmft, in five-year-olds, in total for all of New Zealand, and for the fluoridated and the non-fluoridated areas, was significantly related, to lower socioeconomic status, measured by a socioeconomic status index created from Ministry of Health 2013/14 deprivation quintiles, (% caries-free: $p<0.0001$, $p=0.0030$ and $p=0.0002$ respectively: mean dmft: $p<0.0001$, $p=0.0006$ and $p=0.0001$ respectively, Table 5). No significant relationships were present for Year 8 children (12-year-olds) in total for all of New Zealand, and for the fluoridated and the non-fluoridated areas, were present between dental decay, both percentage caries-free and mean dmft, and lower socioeconomic status, (% caries-free: $p=0.1341$, $p=0.9812$ and $p=0.2371$ respectively: mean dmft: $p=0.0299$, $p=0.6445$ and $p=0.0361$ respectively, Table 5).

Discussion

Although in the F areas compared to the non-F areas there was 1.23–7.34% less dental decay (% caries free and mean dmft) present in the five-year-old children (Table 2) and 6.53–20.18% less dental decay in the Year 8 (12-year-old) children (Table 3), the two-way ANOVA indicates that these differences were not significant (Table 4). In contrast, although dental decay was not significantly affected by the DHB region in the Year 8 children, a significant effect, $p<0.05$ for % caries free and $p<0.01$ for mean dmft, was present in the five-year-old children (Table 4).

Assessment of the effect of socioeconomic status

In 2008, Schluter et al. similarly found no significant relationship between fluoridation status and dental decay in the permanent dentition of 612 nine-year-old children adjusted for the children's age being centred around 9.5 years 2008 (Schluter et al., 2008). In the fluoridated areas the adjusted mean DMFT (95% CI) was 0.21 (0.14, 0.28) while in the non-fluoridated areas the adjusted mean DMFT was 0.20 (0.13, 0.27), $p=0.88$. However, Schluter et al. found

a significantly lower adjusted dmft in the deciduous dentition of the children in the fluoridated area of 1.69 (1.33, 2.04) compared to the adjusted mean dmft in the non-fluoridated area of 2.10 (1.86, 2.33), p=0.02. However, it was noted that a significant SES difference was present between the children in the fluoridated (n=310) and non-fluoridated (n=302) areas. In the fluoridated areas 40% (n=124) were high SES (school decile status 8–10), 41% (n=127) were middle SES (school decile status 4–7) and 19% (n=59) were low SES (school decile status 1–3). For the non-fluoridated areas the corresponding figures were: 19% (n=57) were high SES (school decile status 8–10), 44% (n=133) were middle SES (school decile status 4–7) and 37% (n=112) were low SES (school decile status 1–3). Schluter et al. included weights for the SES strata but the details of how this SES difference is allowed for affects the adjusted dmft. The difference in dmft between the fluoridated and non-fluoridated areas was 0.39 (2.26 – 1.87) before the adjustment and 0.41 (2.10 – 1.69) after the adjustment. If a method similar to that adopted in this paper was used for the adjustment, the $SESI_{Schluter}$ for the fluoridated area would be 0.476 (low SES 19% (decile 1–3, n=59) ÷ high SES 40% (decile 8–10, n=124) and the $SESI_{Schluter}$ for the non- fluoridated area would be 1.965 (low SES 37% (decile 1–3, n=112 ÷ high SES 19% (decile 8–10, n=57). The ratio of the $SESI_{Schluter}$ for the non-fluoridated areas to that for the fluoridated areas is 4.13 (1.965 ÷ 0.476) which is comparable to the value found in the present paper for the ratio of the highest $SESI_{Spittle}$ to the lowest one of 5.91 (2.955 ÷ 0.500). If it was assumed that the spread of SES was the same in the Schluter et al. study as in the present study then 4.13 units of $SESI_{Schluter}$ paper for would be equivalent to 5.91 units of $SESI_{Spittle}$. By measuring the linear regression lines in Figures 1 and 2, it can be calculated that for all New Zealand five-year-olds 1 $SESI_{Spittle}$ unit is associated with a difference of 0.846 dmft and that for all New Zealand Year 8 children (12-year-olds) 1 $SESI_{Spittle}$ unit is associated with a difference of 0.325 dmft. By proportion, assuming a linear reduction in the size of the effect associated with SES with age, at age 9.5 years 1 $SESI_{Spittle}$ unit would be associated with a difference of 0.511 dmft (0.846 – [4.5 ÷7] × [0.846 – 0.325=0.521]). Thus the SES difference in the Schluter et al. paper between the non-fluoridated and fluoridated areas of 1.489 units of $SESI_{Schluter}$, (1.965 – 0.476) corresponding to 2.131 units of $SESI_{Spittle}$ (1.489 × 5.91 ÷ 4.13) would be associated with a dmft difference, at 9.5 years, of 1.09 dmft. If the Schluter et al. figure for the dmft in the deciduous dentition in the fluoridated area of 1.87 was adjusted for SES by adding 1.09, it is likely that, after centralizing for age and allowing for the non-response rate within schools, the resulting dmft for 9.5 year-olds in the fluoridated area, would be closer to 2.50 than 1.69 and that the difference between the fluoridated and non-fluoridated areas for dmft at age 9.5 years would no longer be significant.

In *Our Oral Health: Key findings of the 2009 New Zealand Oral Health Survey*, page 28, after noting that Kanagaratnam et al. (2009) found reduced dental caries in nine-year-olds in fluoridated parts of Auckland, it is noted that another Auckland study by Schluter et al. (2008) of nine-year-olds in Auckland similarly found lower levels of dental caries in children

in fluoridated areas (Ministry of Health, 2010). However, the studies by Kanagaratnam et al. and Schluter et al. are both of the same group of 612 Auckland nine-year-olds (Kanagaratnam et al., 2009; Schluter et al., 2008). Kanagaratnam et al. found that dental caries increased in the deciduous teeth with lower socioeconomic status with the adjusted odds ratios being high-socioeconomic status 1.00 (reference), middle-socioeconomic status 1.56 and low-socioeconomic status 1.96 (Kanagaratnam 2009). The adjusted odds ratio for caries experience (dmfs>0) decreased progressively with increasing exposure to water fluoridation: continuous nonfluoridation 1.00 (reference), intermittent nonfluoridation 0.78, intermittent fluoridation 0.59 and continuous fluoridation 0.42. The ratio of the odds ratio for dental caries for the high-socioeconomic status group compared to the low-socioeconomic group of 0.51 (1÷1.96) is greater than the ratio of the odds ratio for the continuously fluoridated area compared to the continuously nonfluoridated area of 0.42 (0.42÷1) suggesting that fluoridation status has a greater effect on dental caries in five-year-olds than socioeconomic status. This differs from the finding in the present paper. Kanagaratnam et al. noted that no significant relationship was found between residential fluoridation history and dental caries in the permanent dentition. This was attributed, in part, to only some of the permanent teeth being present at the age of nine and it was noted that differences in caries prevalence and severity with differing exposures to fluoride may become more obvious in older children who have more permanent teeth for a longer time.

The efficacy of water fluoridation in under seven-year-olds

In 2011, in a retrospective clinical audit of 1,396 children, aged under seven years, referred for treatment in the decade 2000 to 2009 for comprehensive restorative treatment under general anaesthesia for dental caries, Kamel et al. found that the mean number of teeth with dentine caries was greater in those of low- and medium-socioeconomic status compared with those of high-socioeconomic status and in those from non-fluoridated areas (n=618, water F~0.1 ppm) compared with those from a fluoridated area (n=778, water F=0.85 ppm, Kamel et al., 2013). On average, the children from the non-fluoridated areas were 2.4 months younger and presented with more decayed deciduous teeth than those from fluoridated areas (4.9 and 3.9 teeth respectively, $p<0.0001$). For each tooth type, the mean number of carious teeth at presentation was greater among children from non-fluoridated areas. More decayed teeth were also associated with Maori children (mean number of deciduous teeth with dentine caries in Maori=4.8, non-Maori=4.4), those not living in a high socioeconomic status area (school socioeconomic status group and the mean number of deciduous teeth with dentine caries: low =4.8, p=0.008 compared to high; medium=4.8, p=0.001 compared to high; high=3.9) and those referred for treatment after 2001. The lower mean number of carious deciduous teeth among those presenting more recently was considered to be probably due to the children being referred earlier rather than waiting for marked infection and pain. There was no evidence that more non-European children lived in the non-fluoridated areas. It was concluded that

children with severe dental caries had significantly fewer lesions if they lived in a fluoridated area.

In 1984, in a study of 648 five-year-old Dunedin children, Evans et al. found no significant difference between fluoridated and non-fluoridated areas in the dmft or dmfs of the children in the highest- and middle-socioeconomic status groups while a significant difference was present for the children in the lowest-socioeconomic status group, $p=0.008$ and $p=0.010$ respectively (Evans et al., 1984). Only a relatively small number of children (n=61) living in a non-fluoridated area were studied of whom 15 were in the lowest-socioeconomic group. A mixed effect was found for the effect on dental caries, dmft and dmfs, of socioeconomic status. The middle-socioeconomic status group had significantly more dental caries compared to the highest-socioeconomic status group in the fluoridated area but not in the non-fluoridated area while the lowest-socioeconomic status group had more dental caries compared to the middle-socioeconomic status group in the non-fluoridated area but not in the fluoridated area. The mean dmft scores for the highest-, middle- and lowest-socioeconomic status groups were 1.9, 2.4 and 2.9 respectively in the fluoridated area and 2.7, 3.4 and 6.1 in the non-fluoridated area. In comparison, as shown in Table 2, the mean dmft for five-year-old children for all of New Zealand in 2011 was 1.77 in fluoridated areas and 1.90 in non-fluoridated areas. The children in the study by Evans et al. were examined within a month of their fifth birthday between March 1977 and April 1978. A striking difference is present in the mean dmft in the non-fluoridated areas of Dunedin in 1977–1978 and that the values, both nationally and in Dunedin, approximately 33 years later in 2011. In 1977–1978 the mean dmft in the non-fluoridated areas of Dunedin was 2.7–6.1 compared to the national figure in 2011 for non-fluoridated areas of 1.90 and the figure for the non-fluoridated areas of the Southern District Health Board, including the non-fluoridated parts of Dunedin, in 2011, of 1.11. While a degree of halo-effect might occur with food and beverages produced in fluoridated areas being consumed in non-fluoridated areas, it appears that factors other than water fluoridation have been responsible for the majority of the decrease in dental decay over the 33 year period from 1977–1978 to 2011. It is noted that over 95% of a sample of 465 New Zealand dentists considered that brushing teeth with fluoride toothpaste helps to prevent caries (Grant et al., 2013).

In 1992, Treasure and Dever investigated the prevalence of dental caries in 342 five-year-olds in two fluoridated areas (Ashburton, n=83, and Dunedin, n=64) and two non-fluoridated areas (Oamaru, n=87, and Timaru, n=108) (Treasure and Dever, 1992). The study included examining the effect of socioeconomic status on dental caries in the continuous residents (Ashburton, n=59; Dunedin, n=48; Oamaru, n=67; and Timaru, n=73). The highest- and the lowest-socioeconomic status groups did not differ significantly, at the $p<0.5$ level, in the dmft or dmfs scores between the fluoridated and the non-fluoridated areas while the middle-socioeconomic status group had significantly more dental caries in the non-fluoridated areas compared to the fluoridated areas. The mean dmft scores for the highest-, middle- and lowest-socioeconomic

status groups were 1.05, 0.86 and 1.44 respectively in the fluoridated areas and 1.11, 2.12 and 3.04 in the non-fluoridated areas. The corresponding dmfs scores were 1.58, 1.18 and 2.15 respectively in the fluoridated areas and 1.79, 3.71, and 5.69 in the non-fluoridated areas.

Dental decay and socioeconomic status

Both Evans et al. (1984) and Treasure and Dever (1992) found no significant difference, at the $p<0.5$ level, for the highest-socioeconomic status groups in the dmft or dmfs scores between the fluoridated and the non-fluoridated areas but whereas Evans et al. found a significant difference for the lowest-socioeconomic status group but not the middle-socioeconomic status group in the dmft or dmfs scores between the fluoridated and the non-fluoridated areas, Treasure and Dever found the opposite with their finding a significant difference for the middle-socioeconomic status group but not the lowest-socioeconomic status group in the dmft or dmfs scores between the fluoridated and the non-fluoridated areas. In the 1992 study by Treasure and Dever the difference in dmft for the five-year-olds in the fluoridated area between the highest- and lowest-socioeconomic status groups of 0.39 was greater than the difference between the highest-socioeconomic status groups in the fluoridated and non-fluoridated areas of 0.06. Similarly, the difference in dmfs for the five-year-olds in the non-fluoridated area between the highest- and lowest-socioeconomic status groups of 0.57 was greater than the difference between the highest-socioeconomic status groups in the fluoridated and non-fluoridated areas of 0.21. For the non-fluoridated areas, in the study by Treasure and Dever the difference in dmft for the five-year-olds between the highest- and lowest-socioeconomic status groups of 1.93 was greater than the difference between the highest-socioeconomic status groups in the fluoridated and non-fluoridated areas of 0.06. Similarly, the difference in dmfs for the five-year-olds in the non-fluoridated area between the highest- and lowest-socioeconomic status groups of 3.90 was greater than the difference between the highest-socioeconomic status groups in the fluoridated and non-fluoridated areas of 0.21. This suggests that, as found in the present study, the effects of socioeconomic status are stronger than the effects of fluoridation. Treasure and Dever commented that the children in the fluoridated towns showed only minor variations when the caries data were analysed by socioeconomic status in contrast to the situation in the non-fluoridated areas where there was a marked social gradient with less advantaged children having a higher mean dmft (Treasure and Dever, 1992). Whether the difference was marked for the lowest-socioeconomic group in the non-fluoridated area is questionable as the p value of 0.056 did not quite meet the usual standard for significance of 0.05. However, taken together the Evans et al. and the Treasure and Dever papers suggest that fluoridation is not associated with a significant difference in dental decay in five-year-olds in the highest-socioeconomic status groups but that for the lower- and middle-socioeconomic status groups dental decay is reduced with water fluoridation.

Similarly, in 1994 Treasure and Dever showed in a study of 413 14-year-old children, of whom 227 had been continuously resident in their towns, that there was no significant differ-

ence in dental decay (DMFT and DMFS) between the children of the highest- and middle-socioeconomic status groups living in fluoridated and non-fluoridated areas but that for children of the lowest-socioeconomic group the children there was significantly more dental decay (DMFT, p=0.003 and DMFS, p=0.004) in the areas with discontinued- or non-fluoridation compared to the areas with fluoridation (Treasure and Dever, 1994).

Increased dental decay with lower socioeconomic status was also found by Thomson and Mackay in 2004 (Thomson and Mackay, 2004b). In a study of 388 nine-year-old children for whom complete area- and household-based socioeconomic status data was available there was a consistent pattern whereby children of higher socioeconomic status has lower dmfs and DMFS scores than those of lower socioeconomic status.

The results of the Thomson and Mackay study (2004b) were consistent with those of another 2004 report by Slade et al. in Australia who found, in a study of 5–15-year-old children in Queensland (n=6,704) and South Australia (n=6,814), that children from low-socioeconomic status groups had higher mean dmfs and DMFS values than children from high-socioeconomic status groups ($p<0.01$, Slade et al., 2004).

Further New Zealand studies of dental decay and socioeconomic status

In a 2005 paper, Mackay and Thomson reported a study of 436 nine- and ten-year old children living continuously in a nonfluoridated area (n=183) or continuously (n=137) or intermittently (n=116) in a fluoridated area (Mackay and Thomson, 2005). They found no significant difference to be present in the percentage caries-free or mean dmfs in the deciduous dentition related to exposure to fluoridation up to the age of nine (% caries-free, mean dmfs: with residence in a nonfluoridated area=33.9%, 5.11; with intermittent residence in a fluoridated area=28.4%, 4.29; with continuous residence in a fluoridated area=35.0%, 3.42). For the permanent dentition no significant difference was present for percentage caries-free but there was a significantly lower DMFS ($p<0.01$) with residence in a fluoridated area (% caries-free, mean DMFS: with residence in a nonfluoridated area=54.1%, 1.22; with intermittent residence in a fluoridated area=51.7%, 1.18; with continuous residence in a fluoridated area=64.2%, 0.70). When considering socioeconomic status, the authors found no significant difference to be present in the percentage caries-free or mean dmfs in the deciduous dentition (% caries-free, mean dmfs: low socioeconomic status group=33.3%, 3.79; medium socioeconomic status group =32.2%, 4.54; high socioeconomic status group =32.8%, 4.61). For the permanent dentition no significant difference was present for percentage caries-free but there was a significantly lower DMFS ($p<0.01$) with higher socioeconomic status (% caries-free, mean dmfs: low socioeconomic status group=45.5%, 1.29; medium socioeconomic status group =52.1%, 1.29; high socioeconomic status group =66.7%, 0.74).

The findings by Mackay and Thomson (2005) of no significant difference in 436 Southland nine-year-olds in dental caries in the deciduous dentition between those residing in fluoridated and nonfluoridated areas but a significant difference in the permanent dentition is the

opposite to that found in the 612 Auckland nine-year-olds studied by Schluter et al. (2008) and Kanagaratnam et al. (2009) who found a significant difference in the deciduous dentition but not in the permanent dentition. Similarly, Mackay and Thomson (2005) found no significant difference in the Southland nine-year-olds in dental caries in the deciduous dentition related to socioeconomic status but a significant difference in the permanent dentition is the opposite to that found in the Auckland nine-year-olds studied by Kanagaratnam et al. (2009) who found a significant difference related to socioeconomic status in the deciduous dentition but not in the permanent dentition.

Colquhoun noted earlier in 1987 that results appearing to indicate that dental decay was reduced by water fluoridation were difficult to accept when the socioeconomic status of the non-fluoridated area was less than that of the fluoridated area (Colquhoun, 1987). He found this to be the case when fluoridated (F) Waimairi with an average adult income of $6,947 was compared with the non-fluoridated (non-F) Christchurch urban area with an average adult income of $5,953; when F Balclutha ($,634) was compared with non-F Milton ($5,520); and F Timaru ($6,450) was compared with non-F Oamaru ($6,075). Using average annual income data from the 1981 census and 1984 dental decay data for 12- or 13-year-old children, he found that when similar populations were compared that there was no obvious dental health benefits related to fluoridation. He compared the percentage caries-free and mean DMFT in non-F Christchurch (% caries-free=20.9%, mean DMFT=3.2, average adult income=$6,452) with the values for F Greater Auckland (20.1%, 3.1, $7,272), F Hamilton (15.3%, 3.5, $6,534), F Palmerston North (19.7%, 3.2, $6,982), F Wellington (20.8%, 3.0, $7,413), and F Dunedin (17.7%, 2.9, $6,573).

In a further 2004 study, Lee and Dennison compared dental caries experiences in 8,030 five-year-olds and 6,916 12-year-olds in fluoridated and non-fluoridated areas of Canterbury and Wellington (Lee and Dennison, 2004). They found that caries prevalence and severity were consistently lower for both age groups in the fluoridated areas compared to the non-fluoridated areas. For five-year-olds in the fluoridated areas the dmfs was 2.63 compared to 3.80 in the non-fluoridated areas. The corresponding figures for the DMFS for the 12-year-olds were 1.39 in the fluoridated areas and 3.37 in the non-fluoridated areas. Although the size of the dmfs and DMFS score differences between fluoridated and the non-fluoridated areas were smaller in the highest-socioeconomic status group than in the lowest- and middle-socioeconomic status groups the differences were still statistically significant ($p<0.001$). This is in contrast to the findings for five-year-olds by Evans et al. (1984) and Treasure and Dever (1992) who found no significant difference, at the $p<0.5$ level, for the highest-socioeconomic status groups in the dmft or dmfs scores between the fluoridated and the non-fluoridated areas. Lee and Dennison measured dental decay in 1996 with dmfs and DMFS rather than dmft or DMFT so a direct comparison with the 2011 Age 5 and Year 8 oral health data from the School Dental Services cannot be made. In 1996 the dental health data from the School Dental Service was

grouped in 14 districts while, in 2011 following the establishment of district health boards in 2000 the data was presented for 20 districts. The Canterbury School Dental Service district of 1996 was divided in the Canterbury DHB and the South Canterbury DHB regions. The Wellington School Dental Service district of 1996 was divided into the Hutt Valley DHB and the Capital and Coast DHB regions. However, using the data for percentage caries-free Lee and Dennison found, when their children were examined in 1996, that for the fluoridated areas the percentage caries-free was 60.9% for five-year-olds and 51.7% for 12-year-olds and the corresponding figures in the non-fluoridated areas were percentage caries-free was 54.1% for five-year-olds and 41.6% for 12-year-olds. For comparison, the data for percentage caries-free from the 2011 Age 5 and Year 8 oral health data from the School Dental Services for the fluoridated areas, in the Canterbury DHB, the South Canterbury DHB, the Hutt Valley DHB, the Capital and Coast DHB, and for all of New Zealand, were 53.85% (n=65), 0% (n=0), 60.98% (n=1,348), 74.48% (n=2,633) and 59.91% (n=25,849) respectively for five-year-olds and 39.29% (n=84), 0% (n=0), 59.48% (n=1,488), 66.56% (n=2,512) and 55.17% (n=25,558) for 12-year-olds. The corresponding figures in the non-fluoridated areas were percentages caries-free 63.79% (n=4,745), 58.09% (n=618), 48.61% (n=72), 75.00% (n=16) and 59.18% (n=18,804) for five-year-olds and 56.84% (n=4,748), 51.36% (n=664), 48.00% (n=50), 56.86 (n=51) and 51.79% (n=19,101) for 12-year-olds.

The decline in dental caries in recent decades

Over the 16-year period from 1996 to 2011, the difference in the percentage caries-free between the fluoridated and the non-fluoridated areas, using the figures for Canterbury (Canterbury district of the School Dental Service) and Wellington (Wellington district of the School Dental Service) combined for 1996 and for Canterbury DHB, Hutt Valley DHB, Capital and Coast DHB and all of New Zealand for 2011, has changed from 6.8% in 1996 to –9.94%, 12.37%, –0.52% and 0.73%, respectively, in 2011 for five-year-olds and from 10.1% in 1996 to –17.55%, 11.48%, 9.70% and 3.38%, respectively, for 12-year-olds. No figures were available for making a direct comparison of the percentage caries-free between Canterbury and Wellington in 2011 using the School Dental Service districts that were used to collect the 1996 data.

However, using the 1984 data in the paper by Evans et al. a striking difference is present in the mean dmft for five-year-olds in the non-fluoridated areas of Dunedin in 1977–1978 and that the values, both nationally and in Dunedin, approximately 33 years later in 2011. In 1977–1978 the mean dmft in the non-fluoridated areas of Dunedin was 2.7–6.1 compared to the national figure in 2011 for non-fluoridated areas of 1.90 and the figure for the non-fluoridated areas of the Southern District Health Board, including the non-fluoridated parts of Dunedin, in 2011, of 1.11. Similarly, the mean dmft in the fluoridated areas of Dunedin in 1977–1978 was 1.9–2.9 compared to the national figure in 2011 for fluoridated areas of 1.77 and the figure for the fluoridated areas of the Southern District Health Board, including the fluoridated

parts of Dunedin, in 2011, of 1.01. Decreases occurred in the dmft in five-year-olds in Dunedin between 1977–1978 and 2011 in both the non-fluoridated and fluoridated areas despite the continued absence of fluoridation from the non-fluoridated areas and its having been present in the fluoridated areas for sufficient time for its maximal effect to have become evident. Although Brown in 1988, after considering the Bradford Hill criteria, considered that the evidence was very strong that fluoride had had a major role in the enormous decline in dental decay that had occurred in recent years, these decreases over 33 years in the dmft in Dunedin five-year-olds cannot be attributed to water fluoridation (Brown, 1988).

de Liefde noted in 1998 that when the timing of various forms of fluoride supplementation is correlated with the decline in caries, the decline continues beyond the time of maximum population coverage with fluoridated water and fluoridated toothpaste (de Liefde, 1998). She considered that the convergence of caries prevalence in fluoridated and non-fluoridated areas since the 1970s might require a re-assessment of the fluoride effect. In her view, this convergence, and the overall decline during the past decade without known additional fluoride supplementation, suggested that factors other than fluoride, such as food additives and antibiotics, may have contributed.

Similarly, Colquhoun noted that tooth decay started to decline in New Zealand well before the use of water fluoridation and fluoridated toothpaste commenced and that the decline continued after children had received fluoride all their lives so that the continuing decline could not be because of the fluoride (Colquhoun, 1993).

The importance of socioeconomic status in dental decay

The socioeconomic status index used in the present study was constructed from the deprivation, 2013/14, quintiles, taken from the Ministry of Health website. By using the four of the quintile values in the index, with four degrees of freedom, the value of the fifth quintile was also taken into account. This made more use of the available information on deprivation than using just one quintile would have done, such as using only the value for the most deprived section. The values for deprivation 2013/14 were used because they were the only ones available on the Ministry of Health websites for district health boards and 2013/14 was reasonably close in time to 2011. However, although the study has a large sample size it remains an ecologic study and all the children in each district health board were allocated the same socioeconomic status index rather than this being individualized to each child. With such a blunt index it is notable that a significant relationship was found between the SESI and dental decay in the five-year-old children. The absence of a significant relationship between dental decay and fluoridation suggests that in 2011 socioeconomic status was more important factor for dental decay in five-year-olds than fluoridation status.

Osmunson also found that when applied to the community at large, water fluoridation did not show effectiveness (Osmunson, 2007). When he arranged the 50 USA states and the District of Columbia on the basis of the percentage of their populations that were fluoridated and the

confounding factor of socioeconomic status, he found that approximately 82% of the wealthy and 55% of the poor reported very good to excellent teeth regardless of fluoridation. He considered that using this USA national comparison, evidence for the effectiveness of fluoridation was clearly lacking. Similarly, he found that in 1996, when 46% of the state population in the 39 counties in Washington State, USA, received fluoridated water, a plot of the percentage of the residents in each county receiving fluoridated water versus dental decay rates in third-grade children indicated no evidence of reduction of dental decay with increased fluoridation.

The efficacy of water fluoridation in relation to socioeconomic status

However, as noted in the non-ecologic studies by Evans et al. and Treasure and Dever in which the fluoridation status was recorded individually for each five-year-old child, significantly more dental decay has been found in children of low- or middle-socioeconomic status, but not children of high-socioeconomic status, in non-fluoridated areas compared to fluoridated areas (Evans et al. 1984, Treasure and Dever, 1992). Kamel et al. found similar effects in children less than seven years of age with severe dental caries (Kamel et al., 2013). Those from non-fluoridated areas and those of low- or middle-socioeconomic status had more severe lesions compared to those from fluoridated areas and those of high-socioeconomic status respectively. Lee and Dennison found more dental decay in five-year-olds from all socioeconomic status groups although the size of the dmfs and DMFS score differences between fluoridated and the non-fluoridated areas were smaller in the highest-socioeconomic status group than in the lowest- and middle-socioeconomic status groups (Lee and Dennison, 2004).

The efficacy of water fluoridation in the USA

These findings are consistent with those reported by Yiamouyiannis in his re-analysis of the dental records of the 1986–1987 national survey of USA school children (Yiamouyiannis, 1990). Dental examinations were made on 39,207 children aged 5–17 in 84 areas of which 27 had been fluoridated for 17 years or more (F), 30 had never been fluoridated (NF), and 27 had been partially fluoridated or fluoridated for less than 17 years (PF). No statistically significant differences were found in the decay-free children in the F, NF and PF areas. However, among five-year-olds, the decay rates of deciduous teeth were significantly lower in the F than in the NF areas. To focus on the dft rates among children aged 5–8, the eight areas which commenced water fluoridation between 1970 and 1978 were removed from the PF group and added to the F group. The 5-, 6-, and 7-year-olds in the new F (F*) group had dft rates 24%, 10%, and 10% lower than those of the NF group, respectively, and the dft rate of the five-year-olds in the F* group was significantly lower, $p<0.05$, than that of the NF group. Moreover, among the 5-, 6-, and 7-year-old life-long (continuous) residents in the F* group, the dft rates were 42%, 18% and 11% lower than those of the NF group, respectively, and the dft rate of the five-year-olds in the F* group was significantly lower, $p<0.002$, that that of the NF group. The greater percentage change in the dft rate for the five-years-olds who had been continuous residents in a fluoridated area (42%) compared to those who had not lived all their

life in a fluoridated area 24% supported water fluoridation being the cause of the reduced tooth decay.

Thus, there is evidence from the studies by Yiamouyiannis, Evans, Treasure and Dever, and Lee and Dennison that water fluoridation reduces tooth decay in five-year-old children with the effect being greatest the low- and middle-socioeconomic groups. Different mechanisms have been proposed for the effect (Yiamouyiannis, 1990; Evans et al., 1984; Treasure and Dever, 1992; Lee and Dennison, 2004).

Mode of action of water fluoridation

Featherstone considered that fluoride works primarily via topical mechanisms which include (1) inhibition of demineralization at the crystal surfaces inside the tooth, (2) enhancement of remineralization at the crystal surfaces with the resulting remineralized layer being very resistant to acid attack, and (3) inhibition of bacterial enzymes (Featherson, 1999). He considered that the fluoride in drinking water and fluoride-containing products reduced tooth decay via these mechanisms. In contrast, he stated that the effect of systemically ingested fluoride on caries was minimal and that the level of fluoride incorporated into dental mineral by systemic ingestion was insufficient to play a significant role in caries prevention. He noted the work of Ekstrand et al. which showed that after the ingestion of a fluoride tablet only a transient elevation of fluoride in the plasma occurred and that the levels which appeared in the saliva as a result of a once a day fluoride tablet ingestion were unlikely to have much, if any, topical benefit (Ekstrand et al., 1966). He considered that in the presence of high bacterial challenge, xerostomia or salivary dysfunction, even high levels of topical fluoride might be insufficient prevent the progression of dental caries (Featherstone, 1999).

In a meta-analysis of 20 studies, Griffin et al. found that fluoride prevented caries among adults of all ages (Griffin et al., 2007). They found that any fluoride, self- or professionally-applied or water fluoridation, annually averted 0.29 carious coronal surfaces and 0.22 carious root surfaces with the prevented fraction for water fluoridation being 27% or 0.08 carious coronal surfaces or 0.06 carious root surfaces per year. Yeung also reported the same figures (Yeung, 2007).

Yiamouyiannis considered that water fluoridation may have reduced dft by delaying deciduous tooth eruption (Yiamouyiannis, 1990). He noted that, consistent with this, the dft rate in the F and F* groups reached a maximum later than in the NF group.

For the permanent dentition, Krook and Maylin found that cattle on Cornwall Island with chronic fluoride poisoning with stunted growth and dental fluorosis after exposure to atmospheric fluoride pollution experienced a 1.5–3.0 year delay in permanent tooth eruption because of decreased numbers of the resorbing osteocytes which are required for the preparatory resorption of the roots of the deciduous teeth and of bone (Krook and Maylin, 1979). They noted that their paper showed the cause of the delay in eruption of permanent teeth that had been reported in children in fluoridated communities. In their view, fluoride arrested the

resorption of deciduous tooth roots and of the supporting bone and that the induction of one disease, dental fluorosis, delayed the manifestations of another, dental caries.

The role of thyroid hormone in dental decay

Although the resorption of deciduous tooth roots is not applicable to the eruption of the deciduous dentition, a degree of bone resorption may be. The Cornwall Island cattle had a severe degree of fluorosis and a comparable degree of severe chronic fluoride poisoning has not been described in children receiving water fluoridation. However, stunted growth has been described with higher water fluoride levels than those lived in fluoridation. Ruiz-Payan et al. noted that adolescents using water with 5.3 ppm of fluoride in Villa Ahumada, Mexico, were 5.7 cm shorter in height than those using water with 0.3 ppm (Spittle, 2005). However, even with fluoride levels comparable to those used in water fluoridation more subtle endocrinological changes have been described. Ruiz-Payan et al. found that adolescents in Samalayuca, Mexico, using water with 1 ppm of fluoride had a significant reduction in their T_3 thyroid hormone level to 132±26.8 ng/dL, compared with those using water with 0.3 ppm of fluoride in Ciudad Juarez, Mexico, where the T_3 level was 149.7±20.9 ng/dL (Spittle, 2005; Ruiz-Payan A et al., 2005). Susheela et al. found that children with or even without dental fluorosis from exposure to excess fluoride, either through drinking water or other sources, may have thyroid hormone derangements (Susheela et al., 2005). Low thyroid hormone levels have been linked to delayed tooth eruption (Sturman, 1957; Schuld, 2005). Schuld noted that thyroid hormone deficiency leads to delayed tooth eruption while thyroid hormone excess leads to the acceleration of the tooth eruption. When more fluoride was ingested, it took longer for the teeth to erupt. Rwenyonyi et al. found that the later in life maturation of the enamel was completed, the greater was the severity of dental fluorosis (Rwenyonyi et al., 2000).

Feltman treated 601 pregnant women and 495 of their children through to the age of eight years with fluoride tablets in a dose of 1 mg daily (Feltman, 1956). He noted that many children in the study group showed a marked delay in the eruption of the deciduous teeth which was in some cases a cause for alarm by the parents. The second incisor, second molars, and cuspids were the most frequently delayed, in many cases by as much as a year from the average eruption dates. The delayed teeth, when they did erupt, did so as a group, all within a period of a few days with no evident malocclusion and the jaws and teeth appeared to be normal. Feltman and Kosel noted that the delay in eruption, in some cases by as much as a year from the accepted eruption dates, might be a factor in the lesser incidence of decay (Feltman and Kosel, 1961). They commented that the teeth delayed in eruption had the opportunity to mature more prior to becoming exposed to the forces that trigger the caries mechanism. The eruption pattern of the teeth was considered to be regulated by the thyroid gland (Baume and Becks, 1954). Fluoride was seen to inhibit thyroid function which in turn delayed eruption of the teeth.

Two possible mechanisms for the efficacy of water fluoridation

Thus, although the present ecologic study with 44,653 five-year-olds and 44,659 Year 8 (12-year-old) children showed a significant relationship between dental decay and socioeconomic status but not fluoridation status, it is noted that in some of the literature significantly reduced dental decay has been found in fluoridated areas in five-year-olds, particularly in those of low- or middle-socioeconomic status. Although this reduction in decay has been explained as being the result of a topical action of fluoride on demineralization and remineralization at the crystal surfaces inside the tooth together with inhibition of bacterial enzymes and there is evidence that the ongoing use of topical fluoride may prevent caries among adults of all ages, an alternative explanation for the reduced decay in five-year-olds is that systemically ingested fluoride reduces thyroid hormone levels delaying the eruption of the deciduous dentition and reducing the dmft at five-years because the teeth have been exposed to the caries-inducing oral environment for a shorter time (Featherson, 1999; Griffin et al., 2007).

Susheela et al. found that fluoride in excess may induce diseases normally attributed to iodine deficiency and that the deleterious effects were especially critical in brain tissue if exposure occurred very early in development (Susheela et al., 2005). She found that primary cause of iodine deficiency disorders (IDD) such as low IQ, deaf-mutism and cretinism may not always be iodine deficiency but might be induced by fluoride poisoning and questioned the validity of the fluoridation of drinking water, milk, fruit juices and salt by public health authorities. Lin et al. found that children in an iodine deficient area using water with 0.88 ppm of fluoride had lower IQ scores than children in a low iodine area using water with 0.34 ppm of fluoride (Lin et al., 1991).

Diet and the greater effect of water fluoridation with lower- or middle-socioeconomic status

It is of interest to consider the mechanism whereby higher-socioeconomic status is associated with less dental decay in five-year-olds and why water fluoridation results in a greater reduction in dental decay in lower- and middle-socioeconomic status groups compared to the higher-socioeconomic status group. Thomson et al. note that oral health may be affected by direct environmental effects such as having access to health-promoting environmental exposures such as water fluoridation and indirect effects through having sub-optimal oral hygiene practices and an associated lower exposure to fluoride-containing toothpaste, poorer diet, and lower access to health-promoting exposures such as clinical preventive dentistry (Thomson et al., 2004). Water fluoridation is commoner in cities, with higher housing costs, than in smaller towns where living may be more affordable to those of lower-socioeconomic status. The unidentified factors responsible for the decrease in dental decay in the past 80 years may be commoner in those of higher-socioeconomic status. Colquhoun noted that the decline in decay correlated well with changes in the diet that had occurred (Colquhoun, 1993). He commented that while sugar consumption had remained high, there had been an increase dietary intake of fresh fruit and vegetables, which contained important micronutrients, and of cheese which had decay inhibiting properties.

Price examined many primitive or First Nation people in widespread parts of the world in the early decades of the twentieth century and found they had excellent teeth when they ate their traditional diets (Price, 2006). These diets were diverse and based on sea foods, domesticated animals, game, or dairy products. Some contained almost no plant foods while others had a variety of fruits, vegetables, grains, and legumes. In some, the food was mainly cooked while, in others, many foods, including animal foods, were eaten raw. However, they shared several characteristics such as not containing any refined foods such as white sugar or flour. He found that more vitamins, both fat and water soluble, and minerals were present compared to modern diets. He found that parents who had excellent teeth and facial features on a traditional diet could have children with poorly developed narrow dental arches with crowded teeth, poor development of the nasal passages and the middle third of the face, and marked dental decay when they used a modern diet including white flour and sugar. He noted that the Maoris of New Zealand were:

"… reported by early scientists to be the most physically perfect race living on the face of the earth. They accomplished this largely through diet and a system of social organization designed to provide a high degree of perfection in their offspring. To do this they utilized foods from the sea very liberally. The fact that they were able to maintain an immunity to dental caries so high that only one tooth in two thousand had been attacked by tooth decay (which is probably as high a degree of immunity as that of any contemporary race) is a strong argument in favor of their plan of life."

Nutritionally deficient, refined sugar-rich diets—not a lack of fluoride—are increasingly recognized as the principal cause of continued and even increasing high rates of tooth decay, especially in early childhood, occurring in fluoridated as well as nonfluoridated communities (Burgstahler and Limeback, 2004). A nutritionally balanced diet rich in calcium and phosphorus is necessary for adequate amounts of calcium and phosphate to be present in the saliva.

Those with a lower-socioeconomic status may have less access to an adequate diet with fruit and vegetables. Fluoride toxicity is in part related to it causing oxidative stress and for its treatment Susheela has recommended the daily use of vegetables and fruits as a source of antioxidants, rather than taking drugs or tablets containing nutrients (Susheela, 2007). She lists the antioxidants as vitamin C, vitamin E, carotene, glutathione, quercetin, allicin, capasaicin, ellagic acid, gallic acid, epicatechin, lycopene, glucosinolates, lutein and zeaxanthin. If the anticaries effect of water fluoridation was mediated by fluoride toxicity reducing thyroid hormone levels, then those in the higher-socioeconomic group might be less affected through having a better diet with more antioxidants from fresh fruit and vegetables. Water fluoridation might then have less effect in the higher-socioeconomic group.

Future directions

Peckham has also questioned water fluoridation (Peckham, 2011). He suggested that, given the unknown balance between benefit and harm in relation to water fluoridation, that we

should perhaps be more cautious in pushing forward with further schemes and focus more on developing good oral health strategies that target support for those children and their families who experience the worst dental health. He observed that every complex problem has at least one simple, intuitive, and well presented wrong solution.

Conclusions

The 2011 oral health data indicate that in 2011 dental decay in five-year-old and Year 8 (12-year-old) New Zealand children is not significantly influenced by water fluoridation but that significant increases in dental decay in five-year-olds, but not Year 8 children are associated with lower socioeconomic status. Although other non-ecologic studies in the literature indicate that water fluoridation reduces dental decay in five-year-olds, particularly in low- and mid-socioeconomic groups, there is evidence that this is mediated by impairing thyroid hormone levels with an accompanying risk of systemic toxicity including impairment of brain development.

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SHANTI LAL CHOUBISA

CHRONIC FLUORIDE INTOXICATION IN DIVERSE SPECIES OF DOMESTIC ANIMALS SHANTI LAL CHOUBISA

Abstract

Drinking of fluoridated water for prolonged period causes varying degrees of deformities in teeth and bones of man and domestic animals. These deformities are generally known as dental and skeletal fluorosis, respectively. Other organs or organ systems are also affected by fluoridated drinking water and responsible for genesis of various toxic effects that are included under non- skeletal fluorosis. Prevalence, severity and susceptibility of dental, skeletal and non-skeletal fluorosis was observed in native 2747 mature and 887 immature domestic animals of diverse species living in areas with naturally fluoridated (>1.5ppm F) drinking water. These animals included buffaloes (*Bubalus bubalis*), cattle (*Bos taurus*), camels (*Camelus dromedarius*), donkeys (*Equus asinus*), horses (*Equus caballus*), goats (*Capra hircus*), and sheep (*Ovis aries*). Of these mature and immature animals, 899 (32.7%) and 322 (36.3%) showed evidence of dental fluorosis with varying grades, respectively. In bovines (buffaloes and cattle) dental fluorosis appeared in the form of bilaterally striated and horizontally light to deep yellowish staining on their anterior teeth, but in equines (donkeys and horses), flocks (goats and sheep) and camels it was found to be non-striated, vertically and homogenously. Interestingly, in certain high fluoride endemic areas some immature and mature bovines revealed darker dental staining instead of yellowish colourer. With increasing age, irregular wearing, abrasion of teeth, recession and swelling of gingiva were more prevalent. Polymorphism of dental fluorosis was observed in both mature and immature animals. In general, the maximum prevalence and severity of dental mottling was found in bovines followed by equines, flocks and camels. This indicates that bovines are more prone or highly susceptible to fluoride poisoning as compared to other species of animals.

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In general, those animals that had dental fluorosis were also afflicted with mild to severe skeletal fluorosis characterised with intermittent lameness, wasting of body muscles, snapping sound in legs, periosteal exostoses in the mandibular, ribs, metacarpus and metatarsus regions. In such fluorosed animals histories of colic, intermittent diarrhoea, excessive urination, irregular reproductive cycles, repeated abortions and still births were also found as signs of non-skeletal fluorosis. However, mature animals were found to be severely afflicted with skeletal fluorosis as revealed by its relatively higher (31.2%) prevalence as compared to their counterparts (10.7%). Among different animals species, buffaloes showed the maximum prevalence and severity of osteo- dental fluorosis while it was minimum in sheep. Nevertheless, fluorotoxicosis also varied greatly from species to species and between grass-eaters or grazers (bovines and equines) and plant- eaters or browsers (flocks and camels). Indeed grazers are relatively more susceptible to fluoride in drinking water as compared to browsers due to variation in their food contents or nutrients as well as in their fluoride intake frequency. Other possible factors responsible for acceleration of fluoride toxicosis in animals are also highlighted.

Whatsoever the bovine calves are found to be more sensitive, less tolerant and highly susceptible to fluoride toxicity and also show early signs of fluoride poisoning therefore, they can be considered as ideal bio-indicators for fluoridated water as well as for endemicity of osteo- dental fluorosis. Dental fluorosis can be considered as a bio-marker for fluorotoxicosis in domestic animals.

Keywords: Bio-indicators; Bio-marker; Bovines; Browsers; Camels; Drinking water; Equines; Flocks; Fluoride; Fluorotoxicosis; Grazers; Osteo-dental fluorosis; Susceptibility

Prof. A. K Susheela

HOW FLURIDE TOXICITY AND ASSOCIATED ISSUES AFFECTING MATERNAL AND INFANT HEALTH CAN BE MENDED

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ABSTRACT

Background: Anemia in pregnancy and low birth weight babies, a major public health problem plagues India and several other nations.

***Objective*:** To withdraw fluoride from ingestion from drinking water, food and other sources followed by promotion of a nutritive diet without interfering with iron and folic acid supplementation by the Anti-natal Clinics, and assess its impact on pregnant women and pregnancy outcome.

Methods: Project in two Delhi Government hospitals implemented during 2005-2009; screened 3262 women. Women upto 20 week pregnancy with haemoglobin 11.0 – 5.0 g/dl; with urine fluoride >1.01 mg/l and with no ailment selected. 481 pregnant women grouped into sample (n=234) and control (n=247), through a computerized random sampling procedure. The sample group practiced two interventions; (1) removal of fluoride from ingestion from all sources including drinking water.(2) promoted essential nutrients, micro-nutrients and anti-oxidants through dairy products, fruits and vegetables. Consumption of adequate quantity of carbohydrates and proteins are encouraged too. Control group is not introduced to interventions. But both groups consumed iron and folic acid tablets provided by the hospital and also received routine diet counselling from Anti-natal Clinics. Pregnant women of

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both groups are monitored for urine fluoride level (UFL) and haemoglobin (Hb) until delivery. Body mass index (BMI) recorded initially and prior to delivery. Pregnancy outcome recorded from labor room Register.

***Results*:** In sample group taking into consideration trimester 1 & 2, UFL decreased by 64-68%; increase in Hb in 77%. Babies with ≥ 2.5 kg weight born 79-83%; low birth weight babies <2.5 kg reduced to 21-17%. This is in contrast to, UFL reduced by 47-50%; rise in Hb in 57%; Babies with ≥2.5 kg weight born 57-60%; low birth weight babies reduced to 43-40% in control group. Body mass index improved; Pre-term deliveries and intrauterine deaths in sample group women reduced to an appreciable level compared to control group women.

Conclusion : Anemia in pregnancy and low birth weight babies are an easily rectifiable problem. Greatly reduced intake of fluoride and inclusion of essential nutrients, micro-nutrients and anti-oxidants in the daily diet during pregnancy led to a striking increase in haemoglobin, an improved body mass index, fewer low birth weight babies and reduced number of pre-term deliveries. The benefits accrued through interventions are extraordinary and hitherto unreported. The lessons learned are, a high percentage of anemia in pregnancy is because of adverse reactions of fluoride on the ●gut lining, making it unfit for nutrient absorption, ● gut bacteria destroyed resulting in non-production of Vitamin B_{12} ●RBCs abnormal and short-lived ● under thyroid hormone deficiencies, inadequate stimulus results in low RBC production. Attributing anemia in pregnancy, to undernutrition or mal-nutrition and continue to supplement iron and folic acid tablets without beneficial results and indicating the cause to non-compliance is a gross injustice to pregnant women.

Keywords: Pregnancy – Anemia – Tiny babies – Rectification – Fluoride poisoning – Safe drinking Water – Nutritious diet

ASSOCIATION OF ERK1 POLYMORPHISM AND FLUOROSIS IN COAL-BURNING OF GUIZHOU PORVIENCE

Yan-Jie Liu, Ke-Ren Shan, Zhi-Zhong Guan

Abstract

The extracellular signal regulated protein kinase 1 and 2 (ERK1/2) have identified a important kinase associated with neuronal injury. In our previous study, the ERK1/2 was activated in neurons and brain of rats exposed to fluoride overdose, which has a correlation with the change of brain function. However, whether the multiple single nucleotide polymorphisms (SNPs) of gene ERK1 or ERK2 are connected to the injury caused by overexposure to fluoride is unknown. We therefore conducted ERK1 gene 2 SNPs in 219 persons from an area of coal-burning endemic fluorosis in a case-case study. Both genotype and imputed single nucleotide polymorphisms (SNPs) were included in the analysis. The frequencies of genotype of rs743409 and rs7286558 follow the Hardy Weinberg Equilibrium. We examined 219 cases from the fluorosis district to analyze the association between the genotype of ERK1 gene and dental fluorosis, arthralgia, concentration of fluoride in blood, sex, and age.

We first investigated the association of characteristics of study population and fluorotic symptoms. A statistically significant difference in dental fluorosis was found between male and female. Compared with the reference group, people at age of 30~60 and over 60 years old had more serious dental fluorosis. However, there was no significant tendency for dental fluorosis to be aggravated by concentration of fluoride in blood with a difference being present only in the group with a blood fluoride over 4.0 mg/L compared with the reference group. In addition, all the characteristics sex, age and fluoride in blood could influence the arthralgia.

To evaluate the association between haplotypes of *ERK*1 rs743409 and rs7286558 and the risk of fluorotic symptom, we investigated *ERK*1 genotype and dental fluorosis and arthralgia. The results showed no association between *ERK*1 rs743409 haplotype and dental fluorosis and arthralgia. Nevertheless, the *ERK*1 rs7286558 haplotype was associated with an elevated risk that people were suffered from dental fluorosis, but was not associated with arthralgia. Multiple linear regression was used to analyze the correlation between all covariates and *ERK*1 rs743409 and rs7286558. It was shown that age and fluoride in blood had the most significant effect to dental fluorosis in the standardized partial regression model (P=0.01, P=0.02). The age was associated with an elevated risk that people suffered from arthralgia (Standardized coefficients 0.16 , P=0.02). However, *ERK*1 rs7286558 was associated

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with a protective effect against arthralgia caused by fluorosis. All the genotype of rs743409 and rs7286558 was confirmed by sequencing, and we first found there is a heterozygote in rs7286558 in Han.
The activation of ERK pathway is found in many diseases and some study results indicate the ERK1 is more important than ERK2, and the ERK2 maybe compensate the function of ERK1. Our study indicated that there is interaction effect between ERK 1 gene, fluorotic symptoms and sex or age, which maybe a factor connected to the different degree of symptoms in different persons with fluorosis.

Key words: Fluorosis ; ERK1; Single Nucleotide Polymorphism;

EFFECTS OF LASER-ASSISTED FLUORIDE THERAPY ON ENAMEL DEMINERALIZATION

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Abstract:

The purpose of this *in vitro* study was to evaluate the irradiation efficacy of two types of lasers—either unassisted or assisted by acidulated phosphate fluoride (APF) treatment—on enamel's acid resistance. One hundred and twenty enamel samples, obtained from 20 extracted human molars, were randomly assigned to 6 groups as follows: (1) control (C); (2) exposed to acidulated phosphate fluoride (APF) gel (F); (3) Er,Cr:YSGG laser (EL); (4) irradiated with Er,Cr:YSGG laser through APF gel (EL/F); (5) CO2 laser (CL); and (6) irradiated with CO2 laser through APF gel (CL/F). The specimens were individually demineralized in an acidified hydroxyethylcellulose system, and the acid resistance was evaluated by determining the calcium ion using atomic absorption spectrometry. The results showed that the average concentration of the calcium ion determined in groups C, F, EL, EL/F, CL, and CL/F was, respectively, 3.36, 2.63, 2.26, 2.32, 2.24, and 1.51 ppm. It also revealed that demineralization in the: CL/F group was significantly less than the other groups; and the control group was significantly more than the other groups ($P<.001$). It could be concluded that the effect of CO2 laser irradiation, used with acidulated phosphate fluoride, in decreasing the enamel demineralization was more than all the other groups.

Keywords: Acid Resistance; APF; CO_2 laser; YSGG laser

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BIOPHYSICAL AND BIOCHEMICAL MARKERS OF SKELETAL FLUOROSIS

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Abstract:

Fluoride toxicity is a burgeoning problem worldwide, including in Rajasthan, India. It may be cause serious health problems for both adults and children. Several clinical and experimental studies have reported that fluoride causes bone deformities in skeletal fluorosis but the mechanism of action for this is still unknown. In the present study, 55 male fluorotic patients, aged 32±3.1 years, were selected from the orthopaedic outpatient department at Nims Medical Hospital, Jaipur, India. Age-matched controls were selected from an area where the drinking water fluoride content was less than 1.5 ppm. The subjects and controls were examined for tibial bowing, sabre shin, bow-legs, genu valgum, wider long bone ends with other typical skeletal deformities, bone mineral density, and diaphyseal and metatphyseal changes with calcification and ossification of ligaments. The serum biochemical markers of alkaline phosphatase (ALP), hydroxyproline, osteocalcin, 25-hydroxy vitamin D, calcium, and phosphate were also measured. The results showed the fluorotic patents had high phosphate, ALP, hydroxyproline and osteocalcin, and reduced 25-hydroxy vitamin D and calcium. We concluded that various biochemical parameters can provide a reliable indicator for monitoring the health status of those at risk of fluorosis in high-fluoride endemic fluorosis areas.

Keywords: Alkaline phosphatise; Calcium; Fluorosis; Biochemical markers of skeletal fluorosis; Bone deformities in skeletal fluorosis; Hydroxyproline; Osteocalcin; Phosphate; Skeletal fluorosis; Vitamin D

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Platform Session 6 (Part 1)

Fluoride and the brain

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ALUMINOFLUORIDE COMPLEXES – THE MOST DANGEROUS COMBINATION OF FLUORIDE AND ALUMINUM

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I am grateful to organizers for invitation to share with you my experiences from my study of fluoride as the risk for human health. Today I will speak about the synergy of fluoride plus aluminum and the most dangerous form of fluoride – aluminofluoride complexes (AlF_x).
I will deal with:

1. Introduction.
2. Interactions of AlF_x with G proteins. ·
3. Chemistry of aluminofluoride complexes and phosphoryl transfer reactions.
4. The role of G proteins in cell signaling: G protein coupled receptors.
5. The danger of fluoride and aluminum overload for the human health.
6. Conclusion.
7. References.

1. Introduction

I am from Prague, Czech Republic. At sixties our health experts and authorities were fascinating with the discovery that fluoride in drinking water can prevent tooth decay. Therefore, sodium fluoride was added into tap water and, moreover, pediatricians prescribed pills with it to babies beginning 6 months of their age. When I should give it to my daughter I was already educated in biochemistry and I had the knowledge that fluoride is metabolic poison. Recently we know that fluoride interferes with a number of glycolytic enzymes, resulting in a significant suppression of cellular energy production, such as enolase, lipase, phosphofructokinase, pyruvate kinase, glycogen synthase, succinate dehydrogenase, cytochrome oxidase, various phosphatases, ATP-ases, urease, and cholinesterases, to name a few [1]. The use of fluoride in laboratory investigations helped in the discovery of glycolytic and Krebs-cycle pathways and provided key evidence of fluoride effects on the biochemical and physiological processes.

The argument of fluoride proponents was that the concentration of fluoride in tap water is low (1 mg/l) and that milimolar concentration needed for its biochemical effects on enzymes cannot be reached *in vivo*, inside the cells of human body. Nevertheless, there is a great deal of evidence that the long-term intake of fluoride is harmful for human health.

2. Interactions of AlF_x with G proteins

But back to my story. In 1980s of the last century I was the head of the Department of physiology and my team studied the role of phospholipids in plasma membrane during cell stimulation. In that time it became evident that there is a link between activation of hormonal receptors by the first messenger – hormone – and the generation of so called second messenger. These members were called G proteins. Their name was coined because they bind GDP – guanosine diphosphate in inactive form and to be activated they must exchange GDP for GTP in their alpha part.

In our studies we needed to stimulate G proteins and evoke the breakdown of phospholipids to generate second messengers. However, we had no money to buy expensive agonists and I found that I can use sodium fluoride, which is general activator of all kinds of G proteins. Fluoride was very important tool for the discovery of G proteins. Alfred Gilman and coworkers used fluoride for the adenylyl cyclase activation. Gilman and Martin Rodbell were awarded by the Nobel Prize in Physiology or Medicine 1994 for their discovery of G proteins and their role in cell signaling. Martin Rodbell used the word fluoride 16 times in his Nobel prize lecture! Nevertheless, it was not the fluoride alone, which stimulated G proteins. Gilman noticed already in 1982 that aluminum is a requirement for activation of the regulatory component of adenylyl cyclase by fluoride. The heterotrimeric G proteins are activated when they go from the GDP-bound to the GTP-bound state. The idea that AlF_x acts as a high affinity analogue of the terminal phosphate of GTP was suggested by Bigay and Chabre [see 2].

AlF_x interacts directly with the beta-phosphate of GDP and mimicks the role of the gamma-phosphate. The effect is more readily seen with G proteins because GDP is always tightly bound in the nucleotide site of the protein.

Since that time fluoride plus aluminum traces were used in many laboratories as a tool to investigate G proteins involvement in numerous processes [1].

3. Chemistry of aluminofluoride complexes: phosphoryl transfer reactions

Soluble aluminofluoride complexes – fluoroaluminate (AlF_x) form spontaneously in aqueous solutions containing fluoride and traces of Al^{3+} and appear to act as phosphate analogues. In parallel, the chemistry of aluminofluoride complexes was studied and it was concluded that the most biologically active form is AlF_4, which mimicks phosphate. Aluminofluoride complexes were designed as the molecule of the year 1997.

The phosphate analogue model of AlF_x has been extended to many enzymes that bind phosphate groups. AlF_x can bind to proteins by hydrogen bonds to the fluorine atom just as with oxygen atoms of a phosphate ion. Analogies between phosphate group and AlF_x consist in atomic and molecular similarities. The fluorine atom has the same size and the same valence orbital as oxygen. Aluminum is close to phosphorus; their valence electrons are in the same shell. An Al-F bond is the same length as a P-O bond in phosphate, i.e.1.5 to 1.6 Å. Like phosphorus, aluminum has possible coordination numbers of 1–6, due to the possible hybridization of its outer shell 3p electrons with the 3d orbital. However, an important functional difference between a phosphate group and the structurally analogous AlF_x exists. In phosphate, oxygen is covalently bound to the phosphorus and does not exchange with oxygen from solvent, while in the AlF_x the bonding between the electropositive Al^{3+} and the highly electronegative fluorine is more ionic in character, allowing fluorine in the bound complex to exchange freely with fluoride ions in solution. While the reaction of a bound phosphate with orthophosphate is endergonic and slow, the corresponding reaction with AlF_x is rapid and spontaneous.

Phosphate is a constituent of many important biological substances, including phospholipids, nucleic acids, and several metabolites. Life is truly built around phosphate. Regarding the role of phosphoryl transfer reactions in cell metabolism, we can predict hundreds of reactions, which might be influenced by AlF_x. It seems probable that we shall not find any physiological process, which is not potentially influenced by synergistic action of fluoride plus Al^{3+}. The actual phosphorylation level of a given protein is the result of a delicate balance between kinases and phosphatases.

4. The role of G proteins in cell signaling: GPCRs

G protein-coupled receptors (GPCRs) are the largest family of membrane proteins and mediate most cellular responses to hormones and neurotransmitters, as well
as being responsible for vision, olfaction and taste. At the most basic level, all GPCRs are characterized by the presence of seven membrane spanning α-helical segments separated by alternating intracellular and extracellular loop regions. Individual GPCRs
have unique combinations of signal-transduction activities involving multiple G protein subtypes, as well as G protein-independent signaling pathways and complex regulatory processes. Much of vertebrate physiology is based on GPCRs signal transduction. Heterotrimeric G-proteins act as molecular switches. Regulatory proteins, which acts as GTPase, are selective for binding to the transition state of GTP, which can be
mimicked by GDP bound with the planar ion AlF_4. The picture from recent paper in Pharmacological review demonstrates that AlF_4 is accepted tool for investigation of G proteins and their regulatory proteins.

5. The danger of fluoride and aluminum overload for the human health

It is accepted now that more than 60 % of diseases and health dysturbances are caused by dysfunction of G proteins. Nearly a third of the pharmaceuticals currently on the market target one or more of these receptors. We know from biochemistry and the cell physiology that every extracellular signal is amplified during the cell activation and signal transduction. Moreover, protein phosphorylation constitutes one of the major posttranslational mechanism employed in the physiological regulation of G protein signaling. It means that a few molecules of AlF_x are sufficient to evoke several changes of metabolism and numerous functions. The significant physiological implication brought the observations of additive effect of low AlF_x concentrations with an ineffective hormonal agonist resulting in a maximally effective response. Nevertheless, the impact of such knowledge was not fully recognized.

I was sure that such fascinating knowledge would immediately stop the use of fluoride in tap water and medicine. I tried to publish review articles about this topic. It was Fluoride journal, which accepted our review in 1999, some Czech journals and some monographies on aluminum but we have a long list of top journals, which rejected our articles on AlF_x as a hot potato very quickly. I also submitted a call for the 6th European Framework Programme called European fluoride and aluminum network of Excellence. Over 350 researchers from 17 countries joined this project, unfortunately, it was not accepted by the European commission. I do not want to complain here, I want only demonstrate that the danger of fluoride plus aluminum is not recognized as the hot topic in Europe. It was no wonder in that time because the Belgian ministry of Public Health, Mrs Magda Aelvoet was released in October 2002 since she banned the free sale of supplements with fluoride. Nevertheless I found support from ISFR members, and I am very grateful that namely Niloufer Chinoy joined us in our effort to publish a review article Fluoride interactions: From molecules to disease. It is my compliment to professor Chinoy that she participated in manuscript preparation till her last days.

I would like mention that we combine our effort with researchers from the aluminum field. They are very active in their fight against the increasing amount of biologically available aluminum in the environment, water, and food chains. The most dangerous forms today are the aluminum-adjuvants in several vaccines. Aluminum from vaccines accumulates in human body for a long time and acts as neurotoxicant.

While fluoride in whole organism may not reach concentrations, which were used in the laboratory experiments *in vitro*, there may be moments where fluoride ions reach microenvironments where interference may occur, especially at the active sites of certain enzymes. We also present in our mentioned last review a table, which demonstrates that AlF_x also affects several enzymes and causes many effects primarily attributed to fluoride alone.

6. Conclusion

Neither fluoride nor aluminum were used during evolution of life. These two elements

have no biological functions. However, humans release them into environment, water sources, food chains, and medicine. Soluble aluminofluoride complexes – AlF_x – form spontaneously in aqueous solutions containing fluoride and traces of Al^{3+} and appear to act as phosphate analogs. The discovery of synergistic action of fluoride plus Al^{3+} expanded our understanding of mechanisms of fluoride effects on living organism. The presence of AlF_x has been demonstrated by many studies with crystallized proteins, intact cells, and whole animals. The widespread use of fluoride as a general activator of heterotrimeric G proteins provided evidence that AlF_x is a molecule giving false messages, which are amplified by processes of signal transduction. The phosphate analogue model of AlF_x has been extended to many enzymes that bind phosphate groups. Protein phosphorylation constitutes one of the major posttranslational mechanisms employed in the physiological regulation of G protein-linked signaling. Phosphoryl-transfer reactions are also involved in processes such as regulation of cell metabolism, energy transduction, cytoskeletal protein assembly, regulation of cell differentiation and growth. Considering that all these reactions are fundamental for nearly all biological processes, the common denominator of which is the transfer of a phosphoryl group, we can conclude that fluoride, in the presence of trace amount of Al^{3+}, represents the most dangerous form of fluoride for human health.

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PROTEOMIC ANALYSIS OF HIPPOCAMPUS AND CORTEX IN MICE EXPOSED TO FLUORIDE AND LEAD

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Abstract:
In the environment, fluoride always co-exists with other toxic elements, such as lead. Our previous epidemiological investigation in Datong in Shanxi, China, which is a geologically high fluoride area with lead pollution, indicated that children living in a lead and fluoride co-existent area presented an elevated fluoride and lead body burden, and a reduced IQ. Therefore to study the molecular mechanisms of central neural system injury induced by the above two elements, differently expressed protein spots in hippocampus and cortex in mice treated with 150 mg sodium fluoride/L and/or 300 mg lead acetate/L in their drinking water were detected by two-dimensional electrophoresis and mass spectrometry. The result showed that the 22 identified differentially expressed proteins are mainly related with (1) energy metabolism; (2) cell proliferation and apoptosis signal-related protein; (3) the molecular chaperones; (4) the membrane structure and cytoskeleton-associated proteins; and (5) protein synthesis-related enzymes. The findings could provide potential biomarkers for the lesions in the nervous system induced by fluoride and lead exposure.

Keywords: Cortex; Fluoride; Hippocampus; Lead; Proteomic analysis

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INFLUENCE OF AGE ON FLUORIDE-INDUCED NEUROCHEMICAL AND ULTRASTRUCTURAL CHANGES IN RAT CENTRAL NERVOUS SYSTEM

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Abstract:

Fluoride (F) ingestion is well known to be associated with oxidative stress in experimental animals, but the precise mechanism of its toxicity when associated with advancing age has not yet been delineated. The present study attempted to assess the learning and memory capacity of rats using a water maze test for cognitive functioning. The markers of oxidative stress i.e., reactive oxygen species (ROS), protein carbonyl content (PC), lipid peroxide levels (LPx), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) as well as the metals, Fe, Cu, Zn, and Se were measured in the brain hippocampus of young and aged rats fed with 100 ppm F in drinking water for 90 days. We observed significant changes in the F-treated young and aged rats compared with their respective controls. The lipofuscin content was significantly increased in the F-treated aged rats along with a higher concentration of Fe and with concomitantly lower levels of Zn, Cu, and Se. Ultrastructural studies of the CA1 region in the hippocampus of the F-exposed rats revealed that the changes were more pronounced in the aged F-treated rats with the presence of clustered lipofuscin, vacuolization, lysosomal degradation and nucleolar fragmentation. On the basis of these results, we concluded that F may be linked with neurolipofuscinogenesis and alteration in neurobehavioral activity, and that these changes may be responsible for the development of age-related disorders.

Key Words: Aging; Brain effects of fluoride; Fluoride effects on the aging brain; Neurolipofuscinogenesis; Water maze test.

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FLUORIDE INFLUENCES ALUMINIUM-INDUCED BEHAVIORAL AND BIOCHEMICAL CHANGES IN RAT CENTRAL NERVOUS SYSTEM

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Abstract:

The main route of exposure to the elements fluoride (F) and aluminium (Al) is through the drinking water and both are toxic in excess. Aluminium is extensively used as a flocculent for water purification and dietary and medicinal exposure to aluminium is an important cause of neurodegeneration. In the present study, we investigated the combined effect of F and Al toxicity in the rat central nervous system. We administered to the study animals for 90 days, both separately and in combination, 100 ppm of F in drinking water and 100 mg $AlCl_3$/kg body weight orally. The four groups of animals studied were (i) control group, (ii) F in drinking water group, (iii) F in drinking water + oral $AlCl_3$ group, and (iv) oral $AlCl_3$ group. At the end of the exposure period, the Morris water maze test was carried out and then the rats were sacrificed by anaesthetic overdose. The hippocampus, cerebral cortex, and cerebellum were removed for biochemical investigations: lipid peroxide levels (LPO), protein carbonyl content (PC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), acetylcholinesterase (AChE), superoxide dismutase, and glutathione peroxidase. We observed a higher concentration of serum F in the experimental group as compared to the control group but a reduced serum concentration of F in the Al treated rats. The group given both F and Al showed the maximum deposition of Al in different brain regions along with increased LPO and PC and the maximum decrease in SOD, CAT, and GPx, compared with other groups. A biphasic effect on AChE was seen, with increased AChE activity being found with the combined treatment (F and Al) and with Al treatment but decreased AChE activity in the rats treated with F alone, as compared to the control group. On the basis of these results, we concluded that fluoride increases the deposition of Al in the brain potentiating the changes.

Key Words: Aluminium; Brain deposition of alumininum; Fluoride effects on aluminium deposition in the brain; Fluoride potentiation of aluminium deposition in the brain; Morris water maze test.

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Platform Session 6 (Part 2)

Fluoride and the brain

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THE POTENTIAL ROLE OF FLUORIDE IN AUTISM SPECTRUM DISORDERS

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I will focus in my lecture on the potential contributions of fluoride and aluminum in autism pathophysiology. Over the past several decades the incidence of autism spectrum disorders (ASD) has increased dramatically. A careful review of known environmental and pathological links to ASD indicates that most, if not all, are connected to the immunoexcitotoxic process. While aluminum (Al^{3+}) has been involved among the possible culprits of ASD, fluoride is rarely considered. I will talk about:

1. Prevalence of ASD – autism epidemic.
2. Definition and diagnosis of ASD.
3. ASD etiology; our hypothesis of immunoexcitotoxicity.
4. The potential role of fluoride in the ASD pathophysiology.
5. The potential role of aluminum.
6. The potential role of AlF_x.
7. Conclusions.

1. Prevalence of ASD

Over the past several decades the reported prevalence of ASD has increased dramatically. For example, data from Centers for Disease Control and Prevention (CDC) revealed a nearly fourfold increase between the 1997-1999 and 2006-2008 periods. The prevalence of parent-reported ASD among children aged 6-17 was 2 % in 2012. It means that two of hundred children are affected in the USA. School-aged boys were more than 4 times as likely as school girls to have ASD. Similar prevalence is reported in the UK. There are no complete data from the EU but it seems that the autism prevalence is at least ten to hundred times lower in Europe.

2. Definition and diagnosis of ASD

ASD are a group of neurodevelopmental disorders including autism, childhood disintegrative disorder (CDD), Asperger syndrome, Rett syndrome, and pervasive developmental disorder-not-otherwise specified (PDD-NOS). The terms ASD and autism are often used interchangeably and I also will use them in such manner.

There are two diagnostic systems – the DSM-IV (now DSM-5) used in the USA and ICD-10 (International Classification of Diseases of WHO) used outside the USA, for example in Europe. We compared these two diagnostic systems and it is evident that they have similar symptom criteria for diagnosis of ASD and that much of the ASD increase cannot be explained by improved or different diagnosis.

Both diagnostic systems are based on three general impairments: i) severe developmental deficits in socialization, ii) delayed or abnormal language and communication both verbal and non-verbal, iii) repetitive or unusual behaviors. Behavior of autistic individuals displays ritualistic features, reliance on routines, and impairment of imaginative play.

3. Etiology; our hypothesis of immunoexcitotoxicity

The etiology of ASD remains an unsolved puzzle to scientists, physicians, pediatricians, psychiatrists, and pharmacologists. Of great concern is that no central mechanism has been proposed to explain the various clinical presentations of ASD and no evidence-based therapy has been offered. The heterogeneity of pathophysiological, histological, neurological, biochemical, clinical, and behavioral symptoms provide us little reason to assume that there is one cause of ASD pathogenesis, for example genetic.

Autism appears to be the most highly genetic of the psychiatric disorders, as evidenced by the high risk of autism in additional children in families with an autistic child and the concordance rate for monozygotic twins being much higher than that of dizygotic twins. However, it is evident, that ASD do not follow a simple Mendelian mode of transmission but are clearly a polygenic. The effort of many research teams supported by millions of dollars, did not bring the expected results.

Nevertheless, we suggested in our previous review [1] and in our e-book Cellular and Molecular Biology of ASD [2] that multiple environmental risk factors may cause the dysregulation of immune-glutamate pathways. Blaylock coined the term immunoexcitotoxicity as the possible central mechanism in ASD etiopathogenesis. There is compelling evidence from a multitude of studies indicating that environmental and food borne excitotoxins, such as fluoride, aluminum, glutamate, aspartate, and mercury can elevate blood and brain glutamate to levels known to cause neurodegeneration, brain inflammation, and alterations in the developing brain.

Russell Blaylock was the first who described in his article in Fluoride journal [3] the excitotoxic action of fluoride. Vargas and co-workers reported in their study of autistic persons from

age 5–44 years widespread microglial and astrocytic activation with the most intense within the cerebellum. Microglia are the brain's primary immune cells. Both aluminum and fluoride can activate microglia. Astrocytes are the major site of storage and generation of glutamate, and possibly cytokines.

Consistent with excitotoxicity is the finding of elevated levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and lipid peroxidation products (LPP) in the brain, following fluoride exposure, both *in vitro* and *in vivo*. Also of interest is the finding of elevation in nitric oxide (NO) via induced nitric oxide synthase (iNOS), again a critical component of excitotoxicity. The interactions between excitotoxins, inflammatory cytokines, and disruption of neuronal calcium homeostasis can result in brain changes suggestive of the pathological findings in cases of ASD.

A complete loss of Purkinje cells in cerebellum of autistic persons was observed. Because Purkinje cells are involved in motor coordination, working memory and learning, the loss of these cells are likely to cause symptoms defining behavioral parameters of ASD [for a review see 2].

4. The potential role of fluoride in the ASD pathophysiology

It is remarkable that fluoride is not the key suspicious factor in autism epidemic in the USA. However, exposure to fluoride among infants is a widespread problem in most major American cities. The investigation of the Environmental Working Group of the Fluoride Action Network found that up to 60 % of formula-fed babies in US cities were exceeding the upper tolerable limit for fluoride. Using fluoridated water, a bottle-fed baby will receive up to 250 times more fluoride than from the mother´s milk (http://www.fluoridealert.org).

I mentioned in my previous lecture that fluoride affects a number of metabolic enzymes [4]. Substantial percentages of autistic patients display peripheral markers of mitochondrial energy metabolism dysfunction, such as elevated lactate and alanine levels in blood and serum carnitine deficiency. Fluoride stimulates lactate dehydrogenase (LDH) in hepatocytes and the study Shivashankara et al. (2000) revealed that fluorotic children showed elevated levels of alanine transaminase (ALT). Lactate, the product of anaerobic glucose metabolism in the cytoplasm, accumulates when aerobic metabolism in mitochondria is impaired. Metabolic and mitochondrial defects may have toxic effects on brain cells, causing neuronal loss and altered modulation of neurotransmission systems. Depletion of cellular energy levels increased the vulnerability toward excitotoxins, leading to cell death.

Numerous studies have been published, which have raised the level of concern about the impacts of increasing fluoride exposure on the brain. These studies further highlight that it is not just the teeth, but the brain, that may be impacted by too much fluoride during development.

It is well known that Mullenix and co-workers compared in 1990s behavior, body weight, plasma, and brain fluoride levels after NaF exposures during late gestation, at weaning or in

adults. Rats exposed prenatally had dispersed behaviors typical of hyperactivity, whereas rats exposed as adults displayed behavior-specific changes typical of cognitive deficits.

Reduction of children's intelligence and various psychiatric symptoms, such as memory impairment, difficulties with concentration, and thinking were reported. Several studies appeared from China, which indicated a lowering of IQ in children associated with fluoride exposure. Elevated fluoride content was found in embryonic brain tissues obtained from required abortions in areas where fluorosis was prevalent. These studies showed poor differentiation of brain nerve cells and delayed brain development [for a review see 4]. The fetal blood brain barrier (BBB) is immature and readily permeable to fluoride. Fluoride may belong to the class of developmental neurotoxicants such as arsenic, lead, and mercury. The effects on individuals indicated that for each mg increase of fluoride in urine, a decrease of 1.7 point in full IQ might be expected [4].

These results indicated that chronic fluoride overload in the course of intrauterine fetal life may produce certain harmful effects on the developing brain of the fetus. Significantly more incidences of prematurity and postmaturity at birth of ASD patients was reported by several authors. The recent study of Hart et al. (2009) found more premature births in fluoridated than non-fluoridated upstate New York communities (http://apha.confex.com/apha/137am/ webprogram/Paper197468.html). Prevalence rates in a cohort of 164 families of autistic children referred to The Autism Center at New Jersey Medical School for prematurity were higher than comparable rates reported nationally. It is interesting that the overall prevalence of ASD in New Jersey was the highest among the USA states. It was New Jersey, where the burden of fluoride began during the Second World Ware in the last century due to the uranium production for atomic bomb [5].

The endocrine glands such as the thyroid and the pineal gland, are extremely sensitive to fluoride. It was shown that normal healthy individuals had thyroid function lowered when consuming water at 2.3 ppm. The investigations of Susheela et al. demonstrated that the thyroid gland appears to be the most sensitive tissue in the body to fluoride burden. It is already widely known from Jennifer Luke studies that fluoride accumulates in the human pineal gland [for a review see 4].

5. The role of aluminum

Aluminum (Al^{3+}) has been discussed by several authors among the possible culprits of ASD. Despite the abundance of aluminum in nature, it has no biological function in humans. There is compelling evidence that an accumulation of aluminum in the body appeared recently as the inevitable consequence of the activities of modern human civilization. There are a number of Al^{3+} sources, such as the drinking water, nutrition, cosmetics, and the widespread use of aluminum in medicine, namely in vaccines. It means that humans are not able to avoid exposure to aluminum at present. Wide ranges of toxic effects of Al^{3+} to hundreds of cellular

processes have been demonstrated. Many investigations show that aluminum can elicit impairment of development and immunity, it acts as a hormonal disruptor, neurotoxin, affects cognition and behavior.

Aluminum is used as adjuvant in a wide range of common vaccination. This is one of the reasons why vaccination has been assumed as the possible trigger for ASD [6]. The Al^{3+}-based adjuvants are known as long-lived depots of antigen. These Al^{3+}-adjuvants activate innate immune signals, they are able to appear in the brain and activate microglia. The widely spread response of kids to vaccination known as encephalitic cry is the response to Al^{3+}-induced brain inflammation. It has been demonstrated recently that aluminum nanoparticles remain in the brain for a long time [7].

6. The role of AlF_x

The synergistic action of fluoride and Al^{3+} has the important implication for ASD pathology. Al^{3+} in micromolar concentrations avidly binds with fluoride to form AlF_x. AlF_x has a potency that allows it to activate hundreds of G protein-coupled receptors (GPCRs). This means that the effects of AlF_x result in pathophysiological consequences at several times lower concentrations than fluoride acting alone. Moreover, the effects of AlF_x are amplified by processes of signal transduction. The principle of amplification of the initial signal during its conversion into a functional response has been a widely accepted tenet in cell physiology. It is evident that AlF_x is a molecule giving a false message. Such mechanism could explain the emergence phenomena in etiopathogenesis of ASD on the molecular and cell level. Signaling disorders represent a major cause for the etiopathology of ASD. A number of studies have shown that AlF_x can affect learning and behavior, and induce a loss of cerebrovascular integrity both in experimental animals and humans.

Toxicological potential of fluoride is markedly increased in the presence of trace amounts of Al^{3+}. Varner and coworkers (1998) observed that Al-induced neural degeneration in rats is greatly enhanced when the animals were fed low doses of fluoride. The presence of fluoride caused more Al^{3+} to cross the BBB and be deposited in the brain of rat.

In their study using aluminum fluoride and sodium fluoride, Varner with coworker found damage in the superficial layers of the cortex, amygdala, and cerebellum – all areas endowed with abundant glutamate receptors (GluRs). Others have described a loss of Purkinje cells with chronic fluoride exposure, a cell type containing abundant GluRs. At least two studies have shown that fluoride compounds can activate immune pathways that can lead to, or enhance, autoimmunity. A growing number of studies have shown that inflammatory cytokines and chemokines can markedly enhance excitotoxicity. Moreover, the recent paper of researchers from France and Great Britain [7] demonstrated that cytokines and chemokines help to transport Al^{3+} to the brain.

Microglia also contain numerous GPCRs, which can be affected by AlF_x and we know that

they play an essential role in the development of the brain.

Fluoride and AlF_x affects pineal gland and melatonin

Some symptoms of ASD such as the sleep problems and the early onset of puberty suggest abnormalities in melatonin physiology and dysfunctions of the pineal gland. Many studies indicate clearly that nocturnal production of melatonin is reduced in ASD. Children with the lowest melatonin production had the most neurobehavioral problems. While melatonin is suggested for therapy of autistic children, we recommend sleeping in a dark room. Melatonin is responsible for regulating numerous life processes, including development, immune system, and oxidative stress. Melatonin has been shown to have powerful neutralizing effects on ROS and to increase the levels of several of the antioxidant enzymes in the brain. Melatonin has a gamut of actions in human body. The pineal gland, represented by melatonin, is truly a "regulator of regulators".

Decreased glutathione level

A decrease of glutathione, a major intracellular antioxidant, is one of the best documented biochemical changes in autistic children. When combined with reduced mitochondrial energy production and reduced secretion of melatonin, one can reasonably expect the increase of the vulnerability of neurons and astrocytes to excitotoxicity and oxidative stress. Alterations in metabolites of methionine-homocysteine cycle have been studied in details in ASD and provide a basis for the recommendation of vitamins B_6, folic acid (vitamin B_9), and vitamin B_{12} for the therapy.

7. Conclusions

ASD are highly genetic and multifactorial, with many risk factors acting together. We suggest that the increasing prevalence of ASD during the last decades might reflect the synergistic action of increased burden of new ecotoxicological factors, such as fluoride, aluminum, glutamate, aspartate, mercury, and the increasing number of vaccines in the period of rapid postnatal brain development. According to our hypothesis the key mechanism in ASD pathophysiology is the immunoexcitotoxicity. The epidemiological surveys refer the highest autism prevalence in areas with fluoridated water – USA and UK. Moreover, the high ASD prevalence was found in New Jersey in the third generation!

The long-term fluoride burden has several health effects with a striking resemblance to the ASD. These include hypocalcemia, hypomagnesemia, hypothyroidism, sleep-pattern disturbance, and IQ deficits. Conceivably, fluoride inhibits the release of pineal melatonin. Fluoride interferes with a number of glycolytic enzymes, resulting in a significant suppression of cellular energy production. The synergistic interactions of fluoride plus Al^{3+} increases the potential neurotoxic effect of fluoride and Al^{3+} alone particularly in children, whose brains are uniquely sensitive to environmental toxins.

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Professor Zhi-Zhong Guan

IMPAIRED MITOCHONDRIAL DYNAMICS IN BRAINS, KIDNEY AND LIVER OF RATS WITH CHRONIC FLUOROSIS

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Abstract

In order to reveal underlying molecular mechanism of the mitochondrial dynamics in the pathogenesis of chronic fluorosis, the expressions of mitochondrial fusion and fission proteins, the level of oxidative stress and mitochondrial morphology were investigated in brain, kidney and liver of rats exposed to excessive fluoride. Sixty SD rats were divided randomly into 3 groups, i.e., control group (drinking water containing less than 0.5 mg/l of fluoride, NaF), low-fluoride exposed group (drinking water containing 10 mg/l of fluoride) and high-fluoride exposed group (50 mg/l fluoride). The experimental period was 6 months. The expressions of Mitofusin-1 (Mfn1), Fission-1 (Fis1) and Dynamin-related Protein-1 (Drp1) at protein and mRNA levels were detected by immunohistochemistry and real-time PCR, respectively. The malondiadehyde (MDA) was detected by thiobarbituric acid method; the total antioxidation capability (T-AOC) was analyzed by the colorimetric method. The mitochondrial morphology in cells was observed by transmission electron microscopy. The results showed that in the tissues of brain, kidney and liver of the rats with chronic fluorosis as compared with controls, the protein levels of Mfn1 was obviously decreased, while Fis1 and Drp1 were significantly increased; the expression of the corresponding mRNAs of these proteins were significantly

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changed in accordance with these encoding proteins; the MDA level was elevated and the T-AOC decreased; the abnormal structure of mitochondria was observed. These findings indicate that chronic fluorosis can induce the abnormal mitochondrial dynamics and changed structures in neuronal, renal cell and liver cells, which in molecular mechanism might be involved in high level of oxidative stress resulted from chronic fluorosis.

Keywords: Rats; Fluorosis; Mitochondrial dynamics; Drp1; Mfn1; Fis1; Oxidative stress

Background

In addition to its well-known effects on skeleton and teeth, excessive accumulation of fluoride has been proposed to cause a vast array of symptoms and pathological changes in many other tissues and organs. Numerous investigations have shown that the underlying mechanism(s) may involve elevated levels of free radicals and attenuated antioxidant defenses, i.e., a high level of oxidative stress (Guan et al., 1989; Valavanidis et al., 2006; Gao et al., 2008 and 2009).

Chronic fluorosis has been observed to cause serious damage to mitochondria (Wang et al., 2000; Lee et al., 2008). There are indications that in cultured cells fluoride-induced apoptosis may involve loss of the mitochondrial membrane potential, thereby releasing cytochrome C into the cytosol and leading to apoptotic cell death (Anuradha et al., 2001). Recently, we found that chronic fluorosis disrupts abnormal mitochondrial dynamics in a manner that might lead to a high level of oxidative stress (Luo et al., 2013).

In the present investigation, we evaluated the expression of fission-1 (Fis1), dynamin-related protein-1 (Drp1), and mitofusin-1 (Mfn1), both at the protein and mRNA levels, in the brain, kidney and liver of the rats exposed to chronic fluorosis to characterize the pathophysiological mechanism(s) of mitochondrial damage.

Methods

Sixty Sprague-Dawley (SD) rats (30 males and 30 females, each weighing approximately 90-120 g at the beginning of the experiment) were acclimatized for one week in a well-regulated housing facility (humidity 30-55% and temperature 22-25°C) prior to treatment. These ani% mals were divided randomly into 3 groups of 20 each, i.e., an untreated control group (drinking water naturally containing less than 0.5 mg fluoride/l), a group exposed to a low level of fluoride (drinking water supplemented with 10 mg fluoride/l, prepared with NaF), and a highly exposed group (50 mg fluoride/l). The rats were housed individually in stainless-steel cages suspended in stainless-steel racks and given access to the appropriate drinking water and standard laboratory chow *ad libitum* for 6 months. At the end of the experiment, dental fluorosis, the level of fluoride in the urine, and body weight were determined. These experiments were pre-approved by the regional ethical committee in Guizhou, China.

For detection of the levels of Drp1, Mfn1, and Fis1 protein by immunohistochemistry, following fixation, brain, renal and liver tissues from animals in each group was sliced into 4 μm-thick consecutive sections and immunohistochemiscal analysis performed in accordance with the avidin-bitin peroxidase complex protocol. The sections were first deparaffinized and then incubated with antibodies directed towards Mfn1 (diluted 1:200), Drp1 (1:400 dilution) or Fis1 (1:400) at 4°C overnight. After washing with phosphate-buffered saline (PBS), these sections were incubated with a peroxidase-conjugated polymer carrying antibodies towards rabbit and mouse immunoglobulins. The reactions were visualized with the ChemMateTM DAB$^+$ Chromogen in this same kit. Thereafter, the sections were counterstained with hematoxylin, dehydrated and mounted. As negative controls to assure the antibody specificity, sections were incubated with non-immune antisera.

For measurement of Mfn1, Drp1 and Fis1 mRNA by Real-time quantitative PCR, fallowing isolation of total RNA from the renal tissue using Trizol reagents, 3 μg was converted into first-strand cDNA employing an appropriate kit (Promega, USA) and oligo-d$(T)_{18}$ primers, in accordance with the protocol recommended by the manufacturer. The primers were designed on the basis of the corresponding complete cDNA sequences deposited in GenBank (accession numbers: NM_138976.1 for Mfn1, NM_053655.3 for Drp1 and NM_001105919.1 for Fis1). Real-time qantitative PCR was carried out using the ABI PRISM 7300 Sequence Detection System (Applied Biosystems, USA) in accordance with the manufacture's protocol and analyzed with GeneAmp7300 SDS software. In brief, a 20 μl solution containing 2 μl first-strand cDNA, 2 x QPCR SYBR Green Mix and 1 mM each of forward and reverse primers was subjected to thermal cycling as follows: 10 min at 95°C, followed by 40 cycles at 95°C for 15 sec, and annealing at 70°C for 30 sec. Both RT-PCR and melting curve analysis were routinely performed after amplification to confirm the specificity of this PCR procedure. Relative quantitation of the results of Real-time PCR was achieved employing novel and convenient fluorescent procedure (Zhang et al., 2005) involving a serial dilutions (here, 625-, 125-, 25-, and 5-fold) of any unknown cDNA sample to determine the standard slope. Moreover, this approach was utilized to calculate the median levels of first-strand cDNA, based on the fact that the slope of a traditional standard curve parallels the slope obtained in this manner. The ABI7300 software was used to analyze the data and make calculations.

For determination of the levels of MDA and T-AOC, 10% homogenates were prepared by adding 9 ml normal saline for every g of tissue. The supernatant obtained by centrifugation was used to determine protein with the BCA protein kit. Subsequent steps were performed in accordance with the instructions to the MDA and T-AOC kits. In brief, the level of MDA, one of the products of lipid peroxidation, was quantitated employing the thiobarbituric acid-reactive substance assay (TBARS); whereas, T-AOC was determined by chemical colorimetry and calculated by the following formula: T-AOC (U/mg protein) = OD (control sample)/0.01/30 (Gao et al., 2009).

For electron microscopy, pieces (each approximately 1 mm^3 in volume) of cerebral cortices, renal cortices and liver were fixed with 3% glutaraldehyde for 2 days at room temperature (RT), washed with 100 mM PBS, post-fixed in osmium tetroxide for 2 hrs at RT, and followed by pre-staining in acetate-barbitone for 10 min. Thereafter, the samples were dehydrated in acetone and paraffinized in Epon 812. Ultrathin sections were isolated on nickel fitters, stained with 2% uranyl acetate for 10 min and then with Reynold's lead citrate for 5 min, and finally examined with a transmission electron microscope (Hitachi-7650, Japan) at 60 kV.
The results for the different groups are expressed as arithmetic means±SD. These values were examined for statistically significant differences employing one-way ANOVA utilizing the SPSS18.0 software (SPSS Inc., USA).

Results and Discussion

Obvious dental fluorosis in the form of white or pigmented bands (I°), gray enamel (II°) and even loss of tooth structure (III°) was observed in the rats exposed to excessive fluoride in their drinking water. Moreover, dose-dependent elevation in urinary levels of fluoride and reduced body weight were apparent in these same animals. As expected, the protocol employed here produced successfully an animal model with chronic fluorosis (Lou et al., 2013; Guan et al., 1998).
Integration of the optical density of immunostained cells in the neurons of cerebral cortex, proximal and distal renal tubules and liver cells revealed that the level of Mfn1 protein in the rats receiving excessive fluoride was obviously lower than in the control group; whereas the levels of Fis1 and Drp1 protein were significantly higher. These changes were more pronounced in the group exposed to a high level of fluoride than the low-fluoride group. The levels of Mfn1, Fis1 and Drp1 mRNA in these tissues of rats exposed to fluorosis were significantly reduced and elevated, respectively, in a dose-dependent manner.
Interestingly, mitochondria play key roles in connection with apoptosis, cell signaling, iron metabolism, and steroidogenesis, all of which are related to their dynamic behavior, i.e., the balance between fusion and fission (Karbowski and Youle, 2003; Bereiter-Hahn et al., 2010; Seo et al., 2010; Whelan et al., 2012). When mitochondrial fusion is attenuated by deficiencies in key components involved, such as mitochondrial GTPase-mitofusions (Mfn1 and Mfn2) and optic atrophy type-1 (OPA1), and/or by elevations in the levels of key elements of mitochondrial fission (Fis1 and Drp1), these organelles become fragmented. Conversely, when the balance is shifted towards fusion, the mitochondria will be long and excessively interconnected (Okamoto et al., 2005; Chan et al., 2006; Bereiter-Hahn et al., 2010).
Both Mfn1 and Mfn2 are located on the mitochondrial outer membrane. The N-terminal region contains the guanosine triphosphatase activity, while, like in the case of Fis1, a transmembrane region is present in the C-terminal region. Cells lacking Mfnl or Mfn2 exhibit fragmented mitochondria, which have lost their characteristic tubular structure (Griffin et

al., 2003; Chen and Chan, 2004). Fis1 is also bound to the mitochondrial outer membrane through the transmembrane region located in its C-terminal region. Drp1 is recruited from the cytoplasm to form complexes with Fis1 at potential sites of mitochondrial fission. In cells in which the gene encoding Fis1 has been deleted, formation of this complex is significantly reduced (Mozdy et al, 2000; Tieu and Nunnari, 2000). On the other hand, over-expression of Fis1 can cause fragmentation of mitochondria (James et al, 2003; Yoon et al, 2003). Over-expression of Drp1 or Fis1, which mediates mitochondrial fission, could also induce apoptosis; while inhibition of Drp1 or Fis1 inhibits apoptosis (Lee et al, 2004). Conversely, over-expression of Mfn could promote mitochondria fusion and inhibit apoptosis; whereas inhibition of Mfn promotes apoptosis (Olichon et al, 2003; Sugioka et al, 2004).

In the study, the alterations in the levels of Mfn1 and Fis1 mRNA were almost the same as the corresponding changes in protein levels, indicating that alterations in gene expression may be the primary underlying mechanism.

Mitochondria can be damaged in numerous ways, including morphological disruption, mutations in mitochondrial DNA, restriction of ATP synthesis, imbalances in the steady-state of calcium, accumulation of products that lead to oxidative stress, and metabolic dysfunction.

At present investigation, the level of lipid peroxidation (MDA) in the brain, renal and liver tissues of rats with fluorosis was significantly higher than in the control group, whereas the T-AOC level was clearly lower. Electron microscopic examination of the neurons in brain, epithelia cells in renal tubules and liver cells of the rats with chronic fluorosis revealed enlarged and swollen mitochondria, a vague or absent mitochondrial crest, and mitochondrial division section

Elevated oxidative stress appears to be the primary cause in the symptoms associated with chronic fluorosis (Guan et al., 1989; Stadtman and Levine, 2003; Sargis and Subbaiah, 2006; Gao et al, 2008 and 2009). Increases in free radicals and reduction of antioxidant capacity occur in many organs of rats with experimental fluorosis (Buttke et al, 1994, Guan et al, 1998; Gao et al., 2009).

In our previous investigation, a reduction in the total phospholipid content, a change in the relative amount of polyunsaturated fatty acids (perhaps due to lipid proxidation) and a decrease in the level of ubiquinone (an endogenous antioxidant located in cellular membranes) were detected in the kidney of rats following long-term exposure to high doses of fluoride (Guan et al., 2000). These earlier findings are consistent with the high level of MDA and decreased antioxidant capacity observed here.

Conclusions

In conclusion, attenuated fusion (Mfn1) and enhanced fission (Fis1 and Drp1) of mitochondria is seen in the brain, kidney and liver of rats with chronic fluorosis, which may induce the degeneration of the cells in these organs. These unbalanced mitochondrial dynamics may in

mechanism involve in the enhanced oxidative stress resulted from chronic fluorosis.

Authors' contributions

Professor Zhi-Zhong Guan designed the project, provided the whole research plan and guided the English writing of the manuscript. Dr. Di-Dong Lou carried out the experimental works for rat brain, Shuang-Li Qing for kidney and Yan-Jie Liu for liver. Yan-Jie Liu provided her experiences on the animal model production.

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INVESTIGATION OF INTELLIGENCE QUOTIENT IN CHILDREN AGED 9–12 YEARS EXPOSED TO HIGH AND LOW FLUORIDE IN DRINKING WATER IN WEST AZERBAIJAN PROVINCE, IRAN

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Abstract:

In this study we investigated the relationship between fluoride (F) in drinking water and the intelligent quotient (IQ) in thirty-nine 9–12-year-old children in two areas in West Azerbaijan. The two selected rural areas were very similar with regard to population size, general demographic characteristics, and educational, economic, social, and cultural status but differed in the concentration of F in the drinking water. The subjects were 19 children in the high F region, with a drinking water F of 3.94 mg/L, and 20 children in the low F area with a drinking water F of 0.25 mg F/L. IQ was measured with the Iranian version of Raymond B Cattell test. The IQ of the 19 children in the high F area was lower, mean 81.21±16.17, than that of the 20 children in the low F, mean 104.25±20.73 ($p<0.001$). In the high F area, 57.8% had scores indicating mental retardation (IQ<70) and borderline intelligence (IQ=70–79), while the incidence of mental retardation in the low F area was only 10%. In the low F area, 45 % of children scored as normal or bright normal intelligence compared to only 26.4 % in the high F area ($p<0.001$). The study found that the IQ scores of children residing in an area with a high F level in drinking water were lower compared to those of children in a low F level area. The inverse relationship between F exposure and intelligence of children indicates that exposure of children to high F drinking water increases the risk of impaired intellectual development and a reduced IQ.

Keywords: Cattell RB IQ test; Drinking water fluoride; Fluoride effects on intelligence; Intelligence Quotient; Iran; West Azerbaijan, Iran.

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CORRELATION OF REDUCED PLASMA ACETYLCHOLINESTERASE ACTIVITY AND COGNITIVE IMPAIRMENT IN A FLUORIDE EXPOSED ADULT POPULATION

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Abstract:

Fluoride (F) toxicity is a burgeoning problem worldwide, including in Rajasthan and Karnataka in India. Several clinical and experimental studies have reported that F induces changes in cerebral morphology and biochemistry that affect the neurological development of individuals as well as cognitive processes, such as learning and memory. Several studies have reported an association of high F levels (>1.5 ppm) in drinking-water with lower intelligence. In the state of Rajasthan, almost all districts have high F (up to 18.0 ppm) in their drinking water or ground water sources and about 11 million of the populations are at risk. In the present study, we studied the effect of fluoride on behaviour by selecting 102 male adults from the high F region in the eastern regions of the Jaipur, where the F content in water is 5.5±1.2 ppm. Age-matched controls were selected from the Jaipur district where the F content in water was less than 1.5 ppm. For each group, the serum and urine fluoride levels and the plasma and RBCs acetylcholinesterase activities were estimated and the GHQ60 questionnaire was used to assess behavioral changes. We found the activity of AChE to be significantly ($p<0.001$) reduced in plasma and this correlated with impairment on the GHQ60. The concentration of fluoride in serum was markedly ($p<0.001$) increased. On the basis of these results, with reduced plasma AChE activity and the correlation of the reduced AChE with cognitive changes on the GHQ60, we concluded that it was likely that fluoride exposure causes a deterioration in the functioning of both sympathetic and parasympathetic neurons. However, further in depth studies are required to fully understand the pathophysiology of fluoride neurotoxicity.

Key Words: AChE in plasma and RBCs; Cognitive decline; Fluoride effects on acetylcholinesterase activity; Fluoride in serum; GHQ60.

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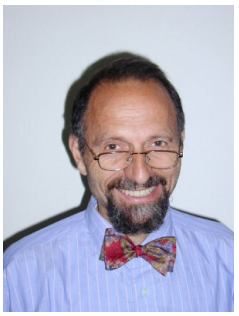
Platform Session 7

Fluoride and the environment



Elian Dahi
Professor of Environmental Engineering

25 YEARS OF DEFLUORIDATION TECHNOLOGY PROJECT

Keynote paper invited at the XXXIst International Society of Fluoride Research,
Tehran 2013 10 18-21

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Abstract:
During the late 1980s and the 1990s comprehensive efforts have been made from Danish and Tanzanian side to investigate and develop a defluoridation technology that is useful and may be sustainable for mitigation of the fluorosis prevailing in affected areas of Tanzania. The Defluoridation Technology Project, DTP, was a main part of these efforts and was based on a decade of collaboration between the Technical University of Denmark, the University of Dar es Salaam, the Ardhi Institute and the Ministry of Water in Tanzania. Danida was the main sponsor of DTP. The achieved results have been reported to Danida and to the Tanzania Commission for Science and Technology and disseminated through numerous presentations, papers, proceedings and international workshops in developing countries and through WHO. This paper summarises some of the DTP outputs: 1) the infrastructural contribution in terms of building and equipping the Ngurdoto Defluoridation Research Station, 2) the research on Magadi occurrence, use and contamination with fluoride, 3) the studies of fluoride uptake by magnesite and magnesite's high capability and its obvious limitations as defluoridation medium, 4) the improvements carried out on the Nalgonda technique, 5) the discovery of contact precipitation and its potential use in future, 6) the project's grand success in developing the char coal furnace as an appropriate and most powerful tool when addressing the problem of fluoride in drinking water in developing countries, and finally 7) the most promising present use of the plain bone char process. It is concluded that what was a mitigation potential 25 years ago is now an opted option for some households and institutions.

1. Introduction

During the late 1980s and the 1990s comprehensive efforts have been made from Danish and Tanzanian side to investigate and develop a defluoridation technology that is useful and may be sustainable for mitigation of the fluorosis prevailing in affected areas of Tanzania. The Defluoridation Technology Project, DTP, was a main part of these efforts and was based on a decade of collaboration between the Technical University of Denmark, the University of Dar es Salaam, the Ardhi Institute and the Ministry of Water in Tanzania. Danida was the main sponsor of DTP[1], [2], [3].

The scientific results of the projects were published in four Ph.D. dissertations [4], [5], [6], [7], fifty-three M.Scs. and pre-M.Sc. fieldworks [2] and in four Proceedings [8], [9], [10], [11], which were made available in the Internet [12].

In this paper some of the DTP contributions and main scientific findings will be summarised.

2. Infrastructural Contributions

The DTP ideas were based on the observation that most the research reported so far was carried out in small laboratory scale using artificially fluoridated water. Further, most of the studies were carried out in Universities in large cities, where fluorosis was not prevalent. Some researchers extrapolated their findings making conclusions about the methods usability in real life situation. Accordingly, in order to allow for more relevant studies, a research station was designed and built in Ngurdoto, where the Nalgonda Water Works already was constructed by the Tanzania Ministry of Water in the middle of a highly fluorotic area.

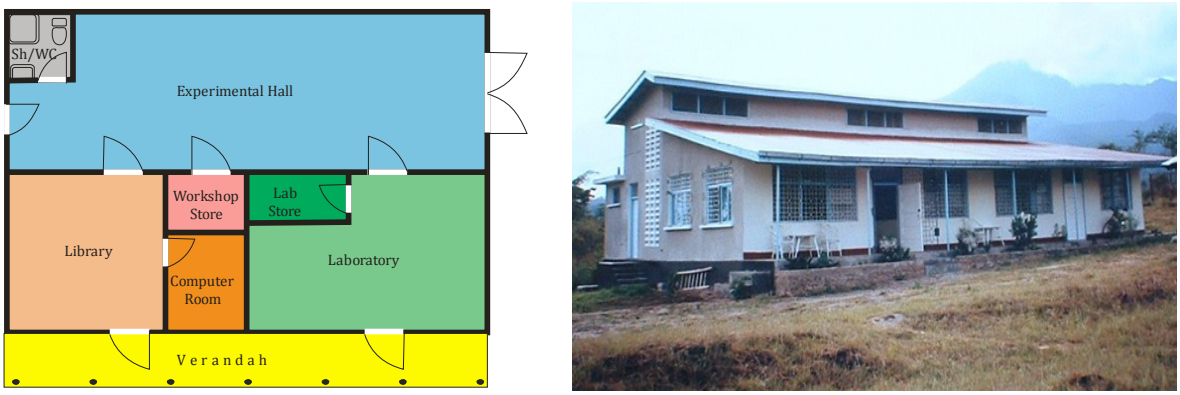

Figure 1. A floor plan and picture of the Ngurdoto Defluoridation Research Hall.

The diagram of Research Station is shown in Figure 1. It shows the main components of the Research Station:

• An Experimental Hall allowing for high elevation of whatever experimental setups needed.

• A Workshop equipped with all kind of tools that may needed for doing maintenance works and building up experimental setups.
• A Laboratory to test most parameters of water quality and also to arrange for continuous titrations and chemo-state experiments.
• A Computer Room that is supported by a powerful generators and battery backups and inverter.
• A Library assisted by a scientific literature database containing titles, authors, addresses and summaries of not less than 400 papers, reports and books.
The research hall, now run by the Tanzania parties, is still the most important platform for fluorotic areas related development in Tanzania.

3. Research Fields

Obviously it is not possible to summarize the DTP research results, which are the output of more than 100 man-year of research work, in a single paper. Instead six topics will be highlighted.

2.1 Magadi Fluoride

Trona, Na2CO3•NaHCO3•2H2O, is a commonly used salt in East, West, and Central Africa, where it is normally mined from the alkaline lakes. The salt product is locally called ***magadi***, a name probably originating from the Masai word ***magad***, meaning bitter. The main use of magadi is cooking tough food products such as beans and maize, utilising its ability to fasten the cooking process and to tenderise and to improve the digestive property of the food [5].

Figure 2. Lake Victoria is not alkaline but it is surrounded by Alkaline Lakes that are a part of the Great Rift Valley.

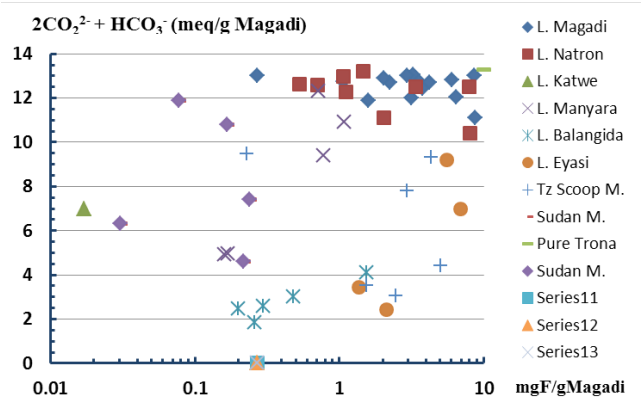

Figure 3. The concentration of fluoride in Magadi from different sources and its content of alkalinity.

The fluoride concentration in the magadi is found to be subject to considerable variation ranging from 0.02 to 8.7 mgF/g Magadi, cf. Figure 1& 2

Analyses of magadi from Tanzania and Kenya show that the samples contain high amounts of fluoride [5], [13], [14], [15], [16]. Thus, the use of magadi that is heavily contaminated with fluoride as a food tenderiser may result in very high fluoride intake, even higher than the "normal" daily fluoride intake of 4 mg/person/day estimated by WHO and the intake through water [17]. Therefore, it may be necessary to purify the fluoride contaminated magadi before using it as a food additive.

A procedure for the treatment in batch system is developed, where 10 ml of water and 1.5 g of bone char are used to treat each g of magadi [5]. Alternatively other magadi sources of low fluoride contamination are likely to be found, e. g. in Sudan and Uganda [18]. The use of magadi in food may contribute significantly to the total intake of fluoride by humans and it may in some cases be more than the intake through water [19]. It is however normally much less than the intake through water in fluorotic areas, cf. Figure 4.

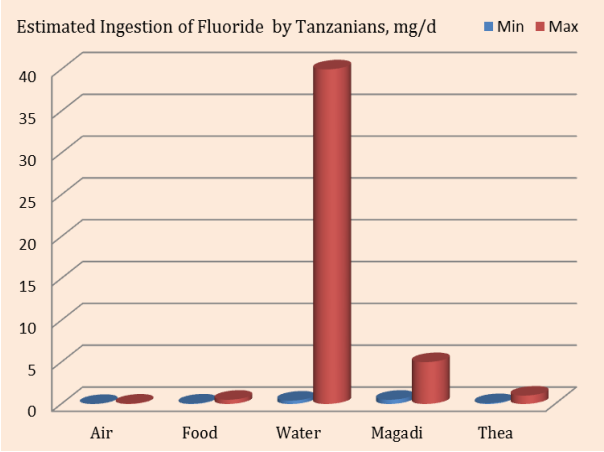

Figure 4. Tanzanians exposure to fluoride through different media, Data from [15].

3.2 Magnesite, Mechanisms & Limitation

Magnesite occurs in mines of Chambogo in the Same district of Tanzania. It consists of almost pure magnesium carbonate, which, if calcined at about 600 ºC, is transformed to magnesium oxide or magnesia, MgO [6].

Singano carried out numerous experiments both in batch and in columns [**6**]. They show that:

• Grained magnesia in batch is able to remove fluoride from water at very high capacity, about 65 mgF/g magnesia.

• However, the removal capacity in column is much lower, about 3.5 mg/g.

• The mechanisms of removal do not match with a simple sorption or chemo-sorption, or with a simple ion exchange.

• The process of removal is kinetically delayed, cf. and controlled by initial magnesia hydrolysis and dissociation:

$X\ MgO_{(s)}$	$+ H_2O$	$\Leftrightarrow (MgO)_{X-1}\cdots Mg(OH)_{2\,(s)}$		
$(MgO)_{X-1}\cdots Mg(OH)_{2\,(s)}$		$\Leftrightarrow (MgO)_{X-1}\cdots Mg(OH)^{+}{}_{(s)}$	$+ OH^-$	

• The hydrolysed magnesia takes up fluoride:

$(MgO)_{X-1}\cdots Mg(OH)^{+}{}_{(s)}$	$+F^-$	$\Leftrightarrow (MgO)_{X-1}\cdots Mg(OH)F_{(s)}$

• The hydrolysed magnesia dissociates further under production of dissolved magnesium ion.

$(MgO)_{X-1}\cdots Mg(OH)^{+}{}_{(s)}$	$\Leftrightarrow (X-1)\ MgO_{(s)}$	$+OH^-$	$+ Mg^{++}$

• Both reaction products are consumed by the water alkalinity and by atmospheric carbon dioxide:

HCO_3^-	$+ OH^-$	$\Leftrightarrow CO_3^{--}$	
CO_3^{--}	$+ Mg^{++}$	$\Leftrightarrow MgCO_{3\,(s)}$	
$CO_2 \quad + OH^-$	$+ Mg^{++}$	$\Leftrightarrow MgCO_{3\,(s)}$	$+H_2O$

• The fluoride is captured mainly by the hydrolysed and dissociated magnesia to form a complex of magnesium-monohydroxy-fluoride:

$X\ MgO_{(s)}$	$+ F^-$	$+ H_2O$	$\Leftrightarrow (MgO)_{X-1}\cdots Mg(OH)F_{(s)}$	$+ OH^-$

• The removal process can only be carried out at a relative high pH, in the range 10.5-11, turning the treated water to be unpalatable.
• Post-treatment of the introduced alkalinity is possible but more costly, more complicated and unrealistic at domestic level in rural areas of developing countries.

3.3 Nalgonda Development

The use of aluminium flocs to remove fluoride from water was studied in USA was studied along with other methods already in 1933. Few years later in 1937 it was concluded that "*the cation associated with the fluoride greatly affects the completeness of its removal by alum flocs and hence this method is not applicable [18]"*. Yet the process was studied thoroughly and adopted by NEERI in India and designated the *"Nalgonda Technique"* after the fluorotic Nalgonda Town where a water-works was erected utilising same technique.
The ministry of Water, Energy and Minerals in Tanzania was assisted by a NEERI specialist to construct a pilot water-works based on same Nalgonda technique in Ngurdoto. The plant was inaugurated in 1990. It implies addition of relatively high amounts of alum and lime as flocculents. Through coagulation, flocculation and sedimentation the fluoride is removed in what is believed to be a co-precipitation process. Addition of 800 mg/L alum and 80 mg/L lime could reduce the fluoride content in the water from 22 mg/L to 3.5 mg/L [19].

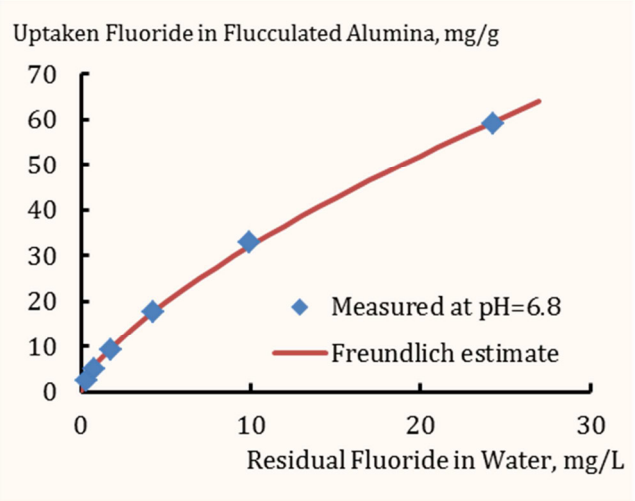

Figure 4. Match of fluoride removal experimentally and theoretically using alum and Freundlich model and where pH is kept at 6.8

The Defluoridation Technology Project focused on smaller scale use of the Nalgonda technique, denouncing the water works setup, due to 1) the high cost of chemicals and 2) the grand environmental implications of highly toxic sludge containing compounds of aluminium and fluoride.

The sorption isotherms of fluoride on flocculated alum was studied and found to follow a Freundlich equation. This resulted in development of a new mathematical equation for the design of chemical dosage, cf. [20]:

$$A = \frac{(S_r - S_t)\cdot V}{\alpha \cdot S_t^{1/\beta}}$$

Where:

A	is the amount of alum required, g.
S_r	is the fluoride concentration in the raw water, mg/L.
S_t	is the residual fluoride concentration in the treated water, mg/L.
V	is the volume of water to be treated in batch, L.
α	is the sorption capacity constant, $L^{(1-1/\beta)} \cdot mg^{1/\beta} \cdot g^{-1}$.
β	is the sorption intensity constant, -.

It was estimated that, for pH = 6.7 and required residual fluoride between 1 and 1.5 mg/l, α = 6 $L^{(1-1/\beta)}$.$mg^{1/\beta}$ .g^{-1} and β = 1.33.

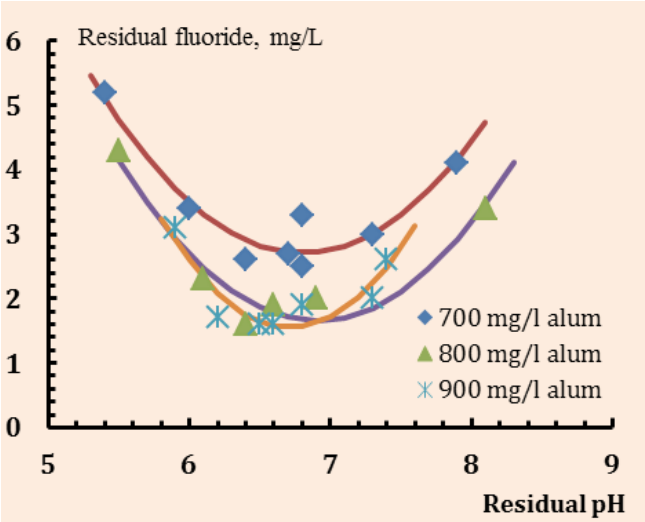

Figure 6. Fluoride removal in batch by adding alum and lime to water containing 12.2 mgF/L.

The amount of lime required is far more difficult to estimate theoretically as it depends on the quality of lime, the alkalinity and pH of the raw water and the fluoride removal itself. Experience however showed that lime addition may be 20-50 % of the alum dosage, in the contrary of what previously recommended in India (5 %) [21]. A pH between 6.2 and 7 would result in maximum possible removal, cf. Figure 6.

It was observed that the use of chemicals by the villagers was easier and safer if the chemicals were packed in plastic bags of different colours.

Further, it was discovered that the captured fluoride is immobilised back to the water if the settled sludge was not removed directly after the sedimentation [21]. Accordingly a new setup based on distribution of the used chemicals in packages and use of bucket system that allows for optimum fluoride removal, cf. Figure 7. As an extra safety measure against potential escape of toxic aluminium and fluoride flocs, additional screening of the treated water is arranged.

This process setup was reliable, simple, affordable and highly acceptable to villagers [22].

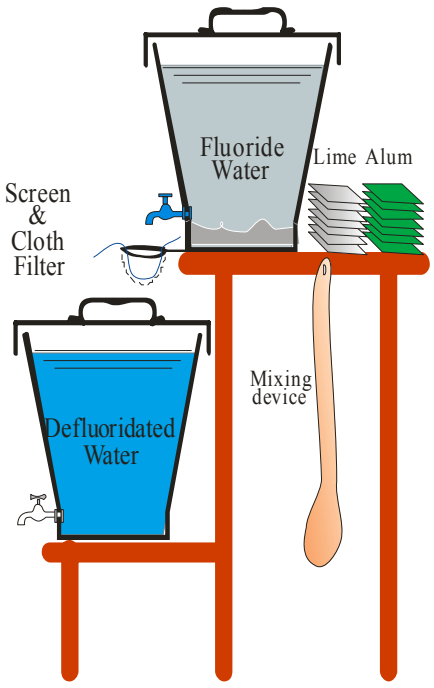

Figure 7. The Nalgonda technique setup as developed for use households.

However, the Nalgonda technique, even in its presented optimised form, could not be advocated for large-scale implementation. Firstly it only results in modest removal efficiencies. The residual fluoride concentration, normally between 1 and 2 mg/L, is low enough to prevent the severe skeletal fluorosis, but still not acceptable compared to what is now believed to be safe concentration in fluorotic areas, i.e. about 0.5 mg/L. Secondly, the process requires very high amounts of chemicals that ad salinity (sulphate) to the water to an extent that water is no longer palatable. Thirdly it adds an extra burden to the household. Finally because of risk of accidental ingestion of escaped toxic aluminium flocs.

Thus the process, which was once reported to be the process of choice in India, completely failed to satisfy the needs in Tanzania.

3.4 Contact Precipitation

The bone char technique has a couple of serious drawbacks. One is that highly fluorotic areas even if determined for using the bone char technique and are fully capable to prepare the bone char locally, can never be self-supplied with respect to bone. The consumption of meat is simply not enough to capture the water fluoride even if all meat bone is collected charred and used in filter columns.

Another disadvantage is that the filter does not run a steady state but as cumulative intermittent process, where the saturation breakthrough point depends on the operation in the individual household. Thus bone char filters need monitoring and action of renewal or regeneration after a specific period of operation. Addition of calcium to the raw water may increase the defluoridation capacity of the bone char, but regeneration or renewal is a must.

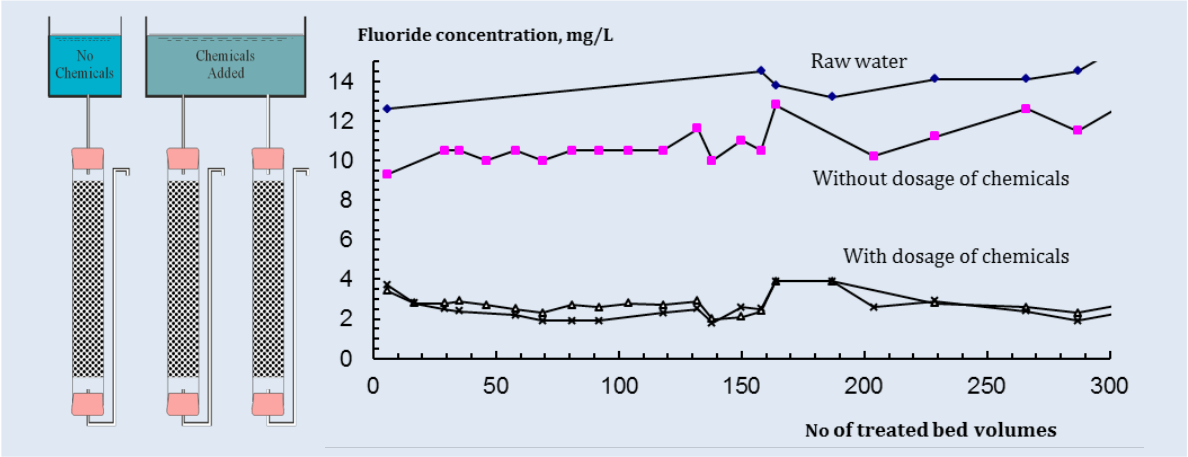

Figure 8. Illustration of the contact precipitation as run in columns. Left triple column setup filled with saturated bonechar and run with and without addition of calcium and phosphate from [23].

It was well known that addition of calcium and phosphate compounds to fluoride water does not result in precipitation of less soluble compounds like calcium fluoride or fluorapatite. However, during the investigation of the bone char regeneration process, it was discovered

that fluoride can be removed almost completely, if calcium and phosphate are added to fluoride water prior to filtration through a bone char filter, even in case the filter is already fully saturated with fluoride [23].

The process was studied in jar test, in manually stirred buckets, in continuously fed columns ,cf Figure 8, and in a "fill, mix and filter" column in the laboratory. Accordingly a pilot plant was constructed in a primary school in Ngurdoto and monitored for a period of two years. The fill, mix and filter technique demonstrated surprisingly high removal efficiencies, 95-98 %, without any sign of break through or saturation, at dosage levels corresponding to calcium/phosphate/fluoride weigh ratio of 8.5/10.8/1.26. The process, which is yet not fully understood, was called contact precipitation [40].

Apparently contact precipitation combines the reliability and the high cost-effectiveness of the Nalgonda technique, with the high efficiency and chemical safely of the bone char technique. However, at the time being the chemicals, though simple and cheap in bulk, are not available in the fluorotic, mostly rural, areas. Then much more research and optimisation and organisational setup is needed, before the contact precipitation can be made superior to the plain bone char technique.

Table 1. Historical events of defluoridation of water at household level.

Year	Country	Worker(s)	Reported
1720	UK	Glass Workers	Crude Hydrofluoric Acid
1771	Sweden	C. W. Sheele	$CaF_2 + H_2SO_4 + Heat \Rightarrow HF$
1809	France	André-Marie Ampère	Pure HF, Named Fluorine
1886	France	Henry Moissan	Electrolysis $HF \Rightarrow F_2$
1893	France	M. A. Carnot	Fluoride Affinity to Bone
1908	USA	Fredrik McKay	Colorado Stain
1916	USA	Unknown	Dental Fluorosis
1931 "	USA "	H. V. Churchill H. V. Smidt et al.	Endemic Fluorosis ∈ Water " " "
1932	DK	I. J. Møller et al.	Skeletal Fluorosis ∈ Cryolite Factory
1935	USA	H. V. Smidt	Lab Defluoridation by Different Media
1937	USA	H. V. Smidt	Water Works Defluoridation Using BC
1942	USA	H. T. Dean	Deans Index
1944	Tanzania	C. J. MacQuillan	Propose Farm Filters in Arusha
1968	New Z.	E. H. Roche	Designed Domestic BC Defluoridator
1975	India	Nawlakhe et al.	Nalgonda Technique
1988	Thailand	P. Phantumvanit et al.	ICOH/WHO BC Defluoridator
1999	Geneva	K. Bailey et al.	DTP/WHO BC Defluoridators

3.5 Bone Char Technology

Already in 1893, while studying fossils, it was reported that bones are able to absorb fluoride [24]. In 1937, immediately after it became known in USA that fluorosis is caused by ingestion of fluoride through drinking water, degreased bones, among other media, were studied at laboratory scale as defluoridation agents [25], [26]. In 1945 "Virgil Bone Black" also called "Animal Char Coal" was commercially available. It was used as decolorizing agent and adsorbent in sugar industries and oil refineries. Burwell [27] suggested to use the processed bone as a defluoridation agent in "small installations", 50 and 100 kg filters, to treat drinking water. Hereafter, in the 50's and 60's, the bone char process was used in larger installations to remove fluoride from municipal water supplies. e. g. in California [28] and Britton [29], [30]. The Britton plant is believed to have been the largest filter ever built. It shifted from using tricalcium phosphate to bone char in 1954. The filter contained 8.5 m^3 for bone char and was treating 5.3 m^3/hour removing its contents of 6.7 mgF/L down to 1.5 mgF/L. Regeneration of the bone char medium was carried out every 1-2 weeks using sodium hydroxide. The plant was operated till 1969. It was closed not in disapproval with the process, but because the water works had to drill a new borehole and new one did not need defluoridation. Prospecting low-fluoride boreholes has since been practised and is possible in areas that are not compactly fluorotic.

Later, in 1968, Roche [31], a medic in New Zealand, proposed a domestic filter, based on bone char, to remove the fluoride from fluoridated drinking water "for those who did not agree on fluoridation of water". It wasn't however before 1988 that a "Defluoridator for Individual Household" was reported and advocated by WHO [32], cf. Table 1 . This defluoridator became widely known as the ICOH defluoridator, cf. Figure 9. The ICOH Defluoridator as launched in 1988., as it was tested and implemented by the Intercountry Centre for Oral Health in Chiang Mai, Thailand [33] and later in India [34] and Tanzania [7].

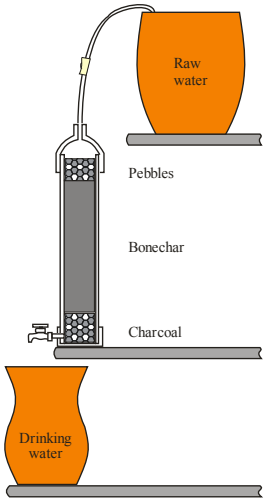

Figure 9. The ICOH Defluoridator as launched in 1988.

Figure 10. The ICOH Defluoridator as tested in India.

The Defluoridation Technology Project, inspired by the ICOH reports, focused on mechanism and the dynamic of the fluoride-bone char uptake. Numerous batch, column and chemo-state experiment were conducted by two Ph. Ds. and many M.Scs. leading to our present understanding of the uptake process. Bregnhøj et al. measure multiple parameters along with the fluoride uptake and do not find that the ion exchange theory experimentally supported. Instead they summarize the uptake as consisting mainly of two processes:

• Initial relatively fast adsorption of fluoride associated with co-sorption calcium ions.

• Secondary relatively slow re-crystallisation in terms of dissolution of bone mineral flowed by precipitation of fluorapatite.

After long contact time, the residual fluoride concentration in the water and the up-taken fluoride in the bone char come to a steady state. Bregnhøj [4] has shown that the relation fits with the classical sorption model of Langmuir, cf. Figure 11.

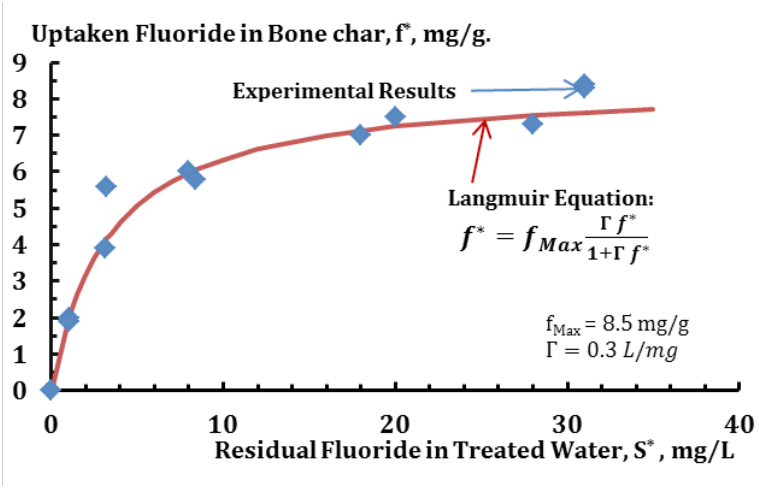

Figure 11. Match of fluoride removal experimentally and theoretically using bone char and the Langmuir sorption model.

Where:

f* is the equilibrium fluoride concentration in the added bone char, mgF/g, $f^* = (S_0\text{-}S^*)/D$.

S^* is the residual fluoride concentration in water, mg/L.

f_{max} is the Langmuir maximum capacity constant, mgF/L.

Γ is the Langmuir intensity constant, L/mg.

D is the dosage of bone char, g/L.

Bregnhøj & Dahi [35] find the deficit to the Langmuir maximum capacity constant at any time, f_{Max}-f, to be decisive to the rate of fluoride removal. The following model was developed to express the removal kinetic in batch:

$$\frac{dS}{dt} = -k_1 \cdot D \cdot (f_{Max} - f) \cdot t^{-0.5}$$

Thus, according to this model, the rate of fluoride removal in a batch system is directly

proportional with dosage of bone char, D, the deficit towards the saturation of the bone char, (f_{Max}-f), and the inverted square root of time. For simplification the concentration of fluoride in the bone char is calculated as f=(S_0-S)/D. By substitution and integration:

$$S = \frac{D \cdot f_{Max} - S_0}{\frac{D \cdot f_{Max}}{S_0} \cdot e^{2(D \cdot f_{Max} - S_0) \cdot k \cdot t^{-0.5}} - 1}$$

In this way it is made possible to describe any fluoride removal in batch by means of only two parameters: a capacity parameter f_{Max}, having the unit mg/g, and a rate constant k, having the unit $L.mg^{-1}.h^{-0.5}$. The model was tested and seems to fit surprisingly well not only with own research results, but also with literature data reported by several workers [35], cf. Figure 12, as well as uptake of fluoride by different media [36].
It has to be mentioned that the capacity and the rate parameters are not universal constants. Rather they are parameters to characterise the different types of bone char, including the processing, i.e. the heat treatment, the crashing and the grain selection.

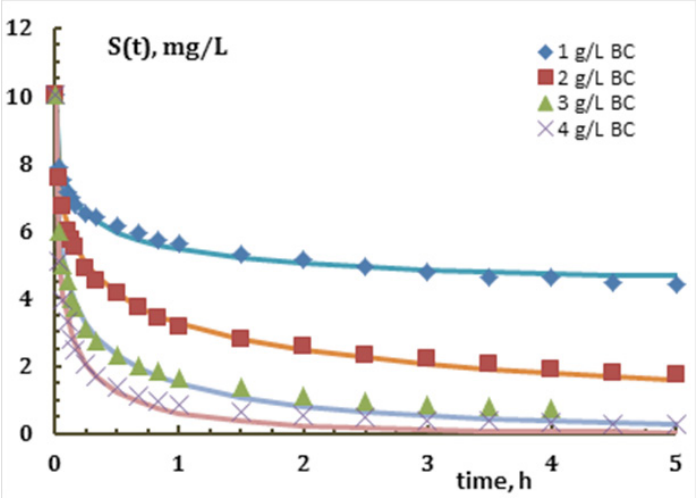

Figure 12. Fluoride uptake on batch chemostate where pH is kept at 7.0. The dots are discrete experimental data while the corresponding curve is given by the kinetic model.

Similarly the fluoride uptake in column was studied and an appropriate model was developed [37]. The model utilizes the kinetics in batch, with the same capacity and rate parameters, but where addition to two purely empirical constants is required. Probably this model could be a useful tool in optimizing filter columns, but because of the complicity of the mathematics of the uptake in columns it has so far only been used to generate concentration profiles and breakthrough curves descriptively plus to provide a qualitative understanding of the uptake in column.

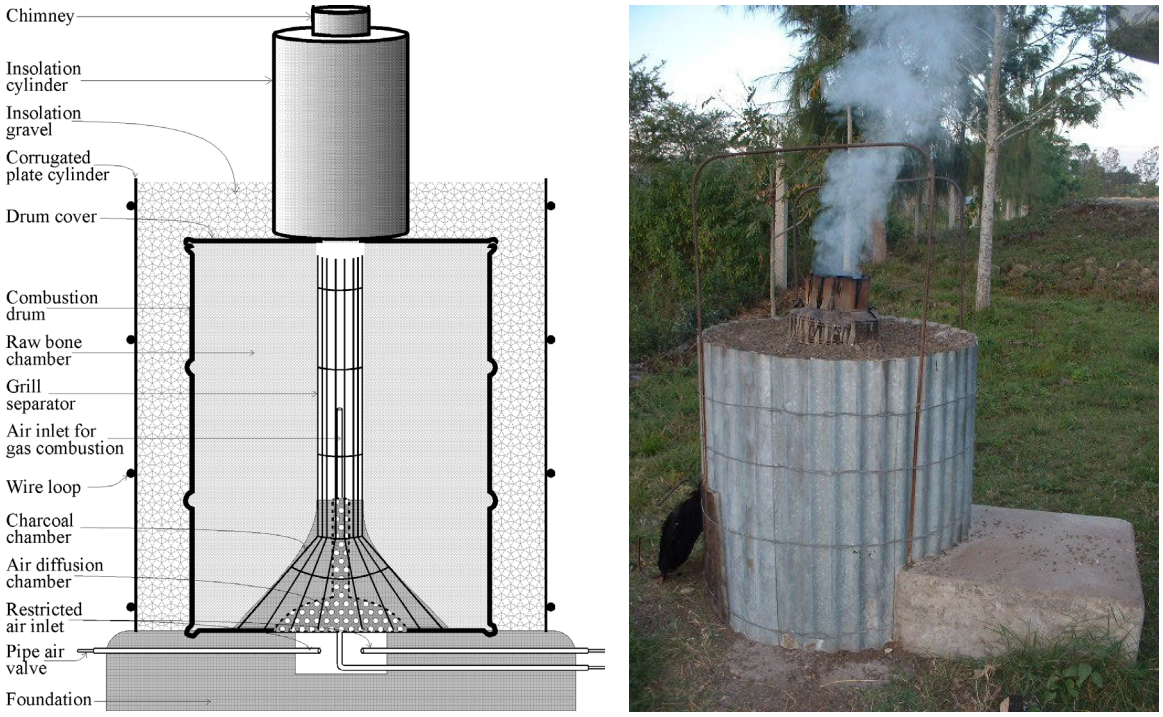

Figure 13. The charcoal kiln for preparation sorption media, a significant development made by the DTP toward appropriate defluoridation of water in developing countries.

3.5 Appropriate Charring Furnace

Because of the observed limitations of the Nalgonda technique, and the high price of imported media, special efforts were put to develop a workable way for local preparation of defluoridation media by heating as required in manufacturing of magnesia, bone char, alumina, clay and the like.

Several known approaches were studied including using computerised electrical furnaces, low tech gas ovens and oil burners in combination with the conventional retort technique for producing bone char. The studied approaches were all less promising, until one of the DTP students, Peter Jacobsen, came up with the new idea of low tech firing of the media in a kiln with charcoal. The idea was promoted and found promising. Several versions of the kiln were developed, ending up with world's largest presently operating kiln installed in the Catholic Diocese Nakuru, Kenya. This kiln can treat 15 tons of bone in a process cycle of 10 days [38]. Some of the advantages of this technique are the low price and the wide availability of the construction and consumption materials in the rural areas. However the construction and especially the operation of the furnace would need experience, as in case of charring fire wood or firing bricks in rural areas. The principles and DTP experiences with the char coal kiln were published in 1997 [39] and recommended by WHO in 2006 [40].

3.6 Filter Design and Implementation

The use of the ICOH defluoridator, Figure 9, was reported to be successful first in Thailand [32] and later in Tanzania [7] and India [34]. In Tanzania Mjengera installed 30 units in

household in the Ketefu Village, where raw water fluoride was 9-15 mg/L. Three different column diameters were used, 80, 100 and 150 mm, length being 550-600 mm. The units were fed with 1.7, 4 and 6 kg of bone char and the units were able to remove the water fluoride down to less than 0.1 mg/L for periods between 6 and 10 months. It was concluded that bone char process was acceptable to the villagers who moreover were motivated to continue using the filter [41]. To the author's knowledge however, the ICOH defluoridators were not maintained on long term basis and no large scale implementation was attempted since [33]. At the time being the Ministry of Water in Tanzania utilises a different filter resembling the filter recommended by Roche [31].

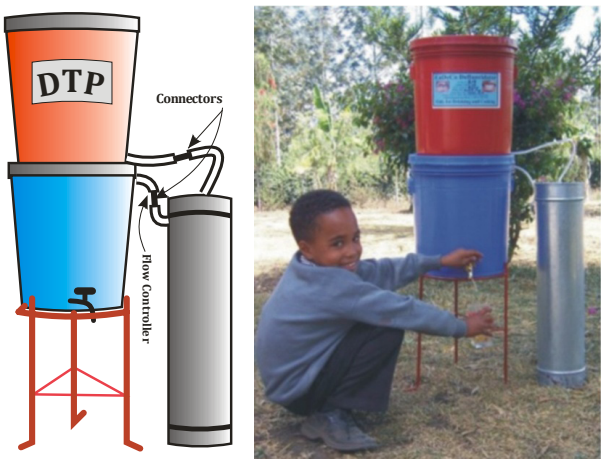

Figure 14. The WHO/DTP advocated Domestic Defluoridator [40].

Like that, during the years, the Defluoridation Technology Project has tested several filters of different configuration. Unfortunately some of the filters, even less promising, were tested in the villages and reported to be acceptable to the villagers [42], [22], [7].

However, as the University Collaboration came to an end and the DTP was requested to summarise its knowledge about defluoridation in a WHO monograph, the project recommended a Bucket-Column-Bucket filter [40], cf. Figure 11. This filter has been installed so far in about 500 households. Another 100 institutional filters were also installed based on same WHO advocated designs [40].

It has not been a DTP objective to make a large scale implantation of the developed technology and realistically stated: we are hardly started in solving the fluorosis problem. Due to the intensive drilling of boreholes at the time being the fluoride problems are at increase in Tanzania. But it is due to the mentioned efforts we now have the tools for mitigation. Thanks to these tools some children, who have been borne in the highly fluorotic region of Arusha, and who have been utilising the developed technology, cf. Figure 14, are now in the teenage with white teeth and healthy skeletons. What was a potential 25 years ago is now an option, and it is opted so far by about 600 motivated households and institutions.

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KINETICS MODELING AND ISOTHERMS FOR ADSORPTION OF FLUORIDE FROM AQUEOUS SOLUTION BY MODIFIED

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Fluoride in drinking water can be either beneficial or detrimental to health depending on its concentration. Its High concentrations lead to dental and skeletal fluorosis and lesions of the endocrine glands, thyroid and liver. This paper describes the removal of fluoride from water using Azolla Filiculoides.

This is an empirical-lab study. The Azolla plant was collected and dried in the oven at 105 in 24 h. it was sieved in 18 – 30 mesh. The optimum values of pH, contact time and adsorbent dosage were determined and different concentrations of fluoride were experimented in lab scale conditions for modified Azolla. The concentration of fluoride was measured in wave-length of 570 nm by spectrophotometer. Also data were fitted with variety isotherm and kinetic models.

The results showed that with increasing of pH of solution, removal efficiency decreased and optimum pH was 5. Also, the removal efficiency of fluoride increased with increasing of adsorbent dose, Contact time and decreasing of initial concentration of fluoride. Adsorption isotherm data showed that the fluoride sorption followed the Langmuir No.1. Kinetics of sorption was well described by pseudo- second order model.

The result showed that modified Azolla Filiculoides can be used successfully as low cost and effective adsorbent for fluoride removal.

Keywords: Fluoride removal, Azolla Filiculoides ,Adsorption kinetic, Adsorption isotherm, Water Treatment

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Dr. Umit Demirel

ARE WE AWARE OF THE FLUOROSIS PROBLEM IN OUR COUNTRIES, INCLUDING TURKEY?

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Abstract

Access to safe and healthy water is a fundamental human right. The contamination of a drinking water source by fluoride can result from either natural or human activities. The community should be informed of the fluoride level in water so that they can collect water for cooking and drinking water from a safe source. Drinking water is typically the largest source of fluoride. Fluoride can also enter public water systems from natural sources, including runoff from weathering of fluoride containing rocks and soils and leaching from soil into groundwater. Fluoride pollution from various industrial discharges and emissions can also contaminate water supplies. However, fluoride may enter the body through the food-chain, drugs, and dental products. Our research indicates that fluoride exposure in parts of Turkey may be at or above the optimal level with the consequent occurrence of fluorosis. However, in spite of this knowledge, plans are underway to continue using fluoride in preventive dentistry. According to the United Nations Development Program (UNDP) and the World Health Organization (WHO), fluorosis is still an endemic public health problem in Turkey, as in other 24 nations. In a recent review, 13 main regions in Turkey were identified as having a fluorosis. In addition, due to a variety of factors, the manifestations of fluorosis differ in different parts of Turkey. Interestingly, these 13 regions are quite far apart from each other. We also estimated the extent of the dental fluorosis problem in two different regions in Turkey. We found a positive association between the fluoride level in drinking water and dental fluorosis. In both regions, the fluoride content in the some drinking water sources was much higher than the average level. The level in the Caldıran district was 0.8– 4.5 ppm and in the Ortakçı and Ericek villages of the Buharkent district it was 2.1– 4.3 ppm. Also, in the same regions, some school children had moderate to high scores for dental fluorosis. The Ministry of Health of

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Turkey collects water samples three-monthly from many points of the country to examine for chemical elements including fluorine. We visited ministerial health authorities and discussed the issue of fluorosis. As a result of many discussions with the authorities, the problem was acknowledged in a ministerial document which stated, "We have noted that some of the water sources contain fluoride above the recommended level".
Studies on fluorosis in the affected areas do not appear to have been done as yet. Unfortunately, the issue of fluoride consumption by people and animals in Turkey has never been adequately addressed in all its dimensions because the studies to date have all been done independently and lack coordination in the discussion of their results. Research on the issue still continues with newer studies continuing to be published but there is still a lack of coordination. We still need to conduct fluoride exposure studies in various areas of the country so that we can substantiate the presence of fluorosis and motivate government officials to take the remedial courses of actions that might be needed.

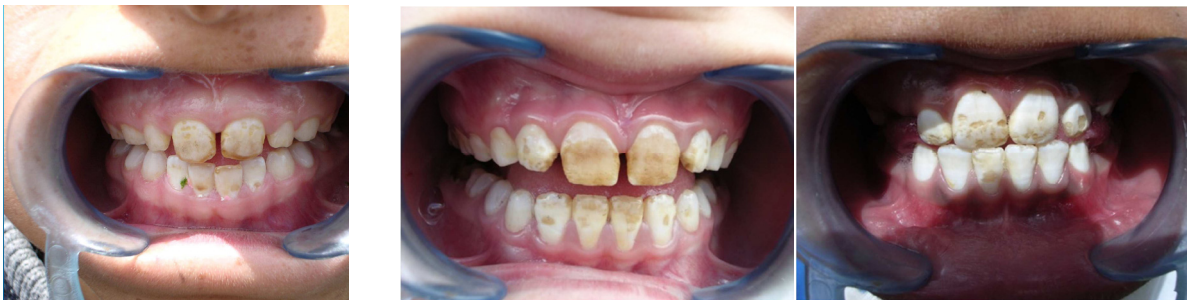
Key words: Fluoride, fluorosis, drinking water, Turkey

Background: Drinking water is typically the largest source of fluoride. Fluoride can also enter public water systems from natural sources, including runoff from weathering of fluoride containing rocks and soils and leaching from soil into groundwater. Fluoride pollution from various industrial discharges and emissions can also contaminate water supplies. However, fluoride may enter the body through the food –chain, drugs, and dental products (1,2,3).According to the United Nations Development Program (UNDP) and the World Health Organization (WHO), fluorosis is still an endemic public health problem in Turkey, as in other 24 nations(4,5).According to a recent scientific review, 13 main regions have been identified as having a fluorosis problem According to this figure; different type of fluorosis problem appears in different parts of Turkey due to a variety of factors. Interestingly, these 13 regions are quite far away from each other (6,7).WHO (World Health Organization) guideline value of 1.5 mg/liter was set for fluoride. All subsequent editions have retained 1.5 mg/liter as the guideline value for fluoride (8, 9).

WHO	1.5 mg/L
Turkey	1.5 mg/L
EC	1.5 mg/L

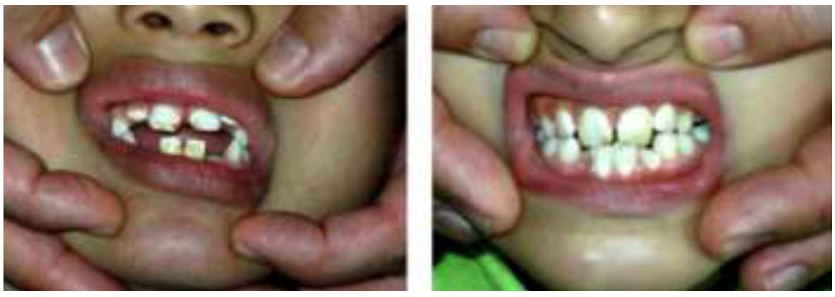
In 2011, The U.S. Department of Health and Human Services (HHS) and Environmental Protection Agency (EPA) proposed the recommendation of 0.7 milligrams of fluoride per liter of water after years. This amount replaces the current recommended range of 0.7 to 1.2 milligrams (10).Also in this condition, many drinking water sources would be risk according to fluoride level.

Methods: We studied to estimate dental fluorosis problem in two different regions in Turkey. We know that volcanic regions can present risks related to fluorosis. Many studies were performed in different volcanic regions concerning fluorosis in Turkey. One of them is the Tendurec volcanic region including Van province Caldiran district where **we studied** the prevalence of **dental fluorosis** among school children by Dean Index.



(Picture 1, U. Demirel's study in the Tendurec volcanic region, Caldiran District).

Geothermal Sources are another source of high fluoride concentrations and have been reported in many areas in Turkey. Turkey has one eighth of the world's geothermal potential and is ranked 7th in the world. One of these areas is the Aydin –Buharkent thermal spring water region where we also studied the prevalence of dental fluorosis among school children by Dean Index.



(Picture 2, U. Demirel's study in the Aydun Province, Buharkent District geothermal source).

The Ministry of Health of Turkey collects water samples three-monthly from many points of the country to examine for chemical elements including fluorine. We visited ministerial health authorities and discussed the issue of fluorosis. As a result of many discussions with the authorities, the problem was acknowledged in a ministerial document which stated, "We have noted that some of the water sources contain fluoride above the recommended level".

Result and Discussion:

After these studies reflected the positive association between fluoride level in drinking water and dental fluorosis.. In both region, fluoride content in the some drinking water sources are

(Caldıran district: between (0,8 - 4,5 ppm) and (Ortakçı and Ericek villages of Buharkent district: between,(2,1 - 4,3 ppm) much higher than the average level. Also in same regions some school children had moderate to higher score according to dental fluorosis problem. Our research indicates that fluoride exposure in parts of Turkey may be at or above the optimal level with the consequent occurrence of fluorosis. However, in spite of this knowledge, plans are underway to continue using fluoride in preventive dentistry.

Figure 3. Data from Ministry of Health of Turkey

Kırıkkale province Keskin district	Sulakyurt, Körkuyu village	2,8 ppm
	Üçevler village	2,68 ppm
Niğde province Ulukışla district	Köşklü village	2,33 ppm
	Altay village	2,37 ppm
Aydın province Buharkent district	Kizildere village	2.15ppm
	Feslek village	4.28 ppm
Bursa province Yenişehir district	Mahmudiye village	2.31 ppm
Denizli province Buldan district	water system	2,6 ppm
Çorum province Merkez district	But village	2.94 ppm
Edirne province Lalabapaşa district	Hanlıyenice village	3,8 ppm

Conclusion:

Studies on fluorosis in the affected areas do not appear to have been done as yet. Unfortunately, the issue of fluoride consumption by people and animals in Turkey has never been adequately addressed in all its dimensions because the studies to date have all been done independently and lack coordination in the discussion of their results. Research on the issue still continues with newer studies continuing to be published but there is still a lack of coordination. We still need to conduct fluoride exposure studies in various areas of the country so that we can substantiate the presence of fluorosis and motivate government officials to take the remedial courses of actions that might be needed.

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USING A GEOGRAPHIC INFORMATION SYSTEM TO IDENTIFY AREAS AT RISK FOR FLUOROSIS IN TAMBON MAE PU KHA AND AMPHOE SANKAMPAENG, CHIANG MAI PROVINCE, THAILAND

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Abstract:
The objective of the study was to create a Geographic Information System which could use various risk factor data, including spatial geographic information, to identify areas at higher risk for fluorosis. Fifteen models were studied with the best being model number 7 which had a correct identification rate of 31.54%. However, as this percentage of less than 50% was relatively low, the model was not considered suitable to define or predict areas that were at risk for fluorosis and further studies to improve the modelling are still required.

Keywords: Amphoe Sankampaeng; Chiang Mai Province, Thailand; Fluorosis risk areas; Geographic Information System modeling; Identifying areas at risk for fluorosis; Tambon Mae Pu Kha; Thailand.

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FLUORIDE EXCESS IN GROUND WATER OF DUNGARPUR (INDIA): SEASONAL FLUCTUATION AND CLINICAL MANIFESTATIONS

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ABSTRACT

Dungarpur district located in the southern part of the state of Rajasthan in India is one of the noted regions in country falling in the category of being an endemic zone of fluorosis, a disease condition due to an excess of fluoride content in the drinking water. The reason behind the excess fluoride in the water is the presence of geomorphologic bearing rocks like granite and granite – gneisses.

We surveyed the drinking water sources of 49 selected villages and analyzed their fluoride content and other physicochemical parameters. Sampling was conducted twice in the year, once during rainy season and for a second time during post monsoon. The fluctuations in values were assessed and data were compared with two earlier surveys. Seasonal and temporal variations in the fluoride content of the sampled drinking water were recorded. Statistical analysis revealed moderate correlation between fluoride and TDS in many pockets. Since the fluoride content is usually related to ultra-basic rock in the aquifers, in some pockets correlation between alkalinity and fluoride content was also noted.

As per some earlier reports (Choubisa, 2001, 2010), in high fluoride pockets of the district cases of dental and skeletal fluorosis were very prevalent among men and domestic animals. Some recent papers recount the symptoms experienced by various domestic animals with fluorosis. There is some evidence that animals which feed on fodder rich in calcium and ascorbic acid have some protection from fluoride toxicity symptoms (Choubisa et. al. 2011). Some model mitigation programs on this endemic problem have also been conducted by some agencies.

Key words: Ground water, fluoride content fluctuation, fluorosis, human, domestic animals, Dungarpur, Rajasthan

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Abstracts
Poster Presentations

DETERMINATION OF FLUORIDE CONCENTRATION IN WATER AND BREAST MILK IN GOLESTAN PROVINCE, IRAN

H. Faraji[1], N. Vakili Saatloo[2], A. Mahvi*[1], B. Akbari[3], G. Lashkarboloki[4]
Tehran and Bandar Gaz, Iran

Abstract:
The purpose of the present study was to quantify the amount of fluoride (F) in breast milk through the standard F ion-selective electrode method in two regions of Golestan province, Iran, with different water fluoride concentrations (Bandar Gaz 0.4–0.5 ppm and Nokandeh 0.6–0.7 ppm). The mean and standard deviation for the F concentration in 20 human milk samples were 2.067±0.310 µg/L for Bandar Gaz (n=12) and 2.293±0.838 µg/L for Nokandeh (n=8). No significant relationship was found between the fluoride concentration in the drinking water and that in human breast milk ($p>0.05$).

Keywords: Breast milk; Fluoride in breast milk; Fluoride in water; Golestan province, Iran.

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EVALUATION OF FLUORIDE CONTENT OF GROUNDWATER RESOURCES AND THE ROLE OF GEOLOGIC FORMATIONS IN KHOSUYE-HAJIABAD PLAIN, ZARRINDASHT, FARS PROVINCE, IRAN

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Abstract

Endemic dental fluorosis within some of the Khosuye-Hajiabad plain residents led the authors to investigate fluoride content of groundwater in the area. In this study, three drinking water wells located at Galugah, Chahsabz and Hajitahere areas in the Khosuye-Hajiabad plain, Zarrindasht, were sampled twice during 2011-2012. Different hydrogeochemical parameters of the water samples such as cation and anion contents, especially fluoride, were measured. The results show fluoride content of higher than WHO standard in some of the samples which can explain the incidence of fluorosis in the area. Fluoride content variations in the waters and its correlation with other ions suggest a relavant role of exposed geologic formations in each area in increasing the fluoride content of the waters. Accordingly, the probable sources of fluoride in ground waters in Galugah area are gypsiferous formations such as Gachsaran and Mishan, in Chahsabz area they are calcareous formations such as Asmari-Jahrom, and in the Hajitahere area they are the Hormoz salty formation. Thus topographic conditions, fault operations, and the distance from exposed formations have different roles in each of the areas studied.

Keyword: Fluoride, Drinking water, Fluorosis, Geologic formations, Zarrindasht, Fars

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DETERMINATION OF FLUORIDE CONCENTRATION IN WATER AND BREAST MILK IN GOLESTAN PROVINCE, IRAN

H. Faraji[1], N. Vakili Saatloo[2], A. Mahvi[*1], B. Akbari[3], G. Lashkarboloki[4]
Tehran and Bandar Gaz, Iran

Abstract:
The purpose of the present study was to quantify the amount of fluoride (F) in breast milk through the standard F ion-selective electrode method in two regions of Golestan province, Iran, with different water fluoride concentrations (Bandar Gaz 0.4–0.5 ppm and Nokandeh 0.6–0.7 ppm). The mean and standard deviation for the F concentration in 20 human milk samples were 2.067±0.310 µg/L for Bandar Gaz (n=12) and 2.293±0.838 µg/L for Nokandeh (n=8). No significant relationship was found between the fluoride concentration in the drinking water and that in human breast milk ($p>0.05$).

Keywords: Breast milk; Fluoride in breast milk; Fluoride in water; Golestan province, Iran.

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INVESTIGATION OF FLUORIDE DISTRIBUTION PATTERN IN GHIR PLAIN AQUIFER, FARS PROVINCE, IRAN

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Abstract:
In this study, 10 wells (4 urban and 6 rural drinking water resources) of Ghir city, Fars province, and its surrounding villages respectively were sampled in May 2013 to investigate the probable cause of endemic dental fluorosis in the area. Fluoride concentration and some other hydrogeochemical factors were measured in the samples. Results show 60% of the samples have fluoride content higher than the WHO permissible limit of 1.5 mg/L. Fluoride concentration map drawn by Arc GIS software shows thedistribution pattern of fluoride in the Ghir plain aquifer. In the map Tang-e-Rueen and Dehbeh villages have the highest and lowest fluoride contents respectively. Consideration of the widespread exposure of calcareous formations in the former area suggests the probable role of this type formation in increasing the fluoride content of ground waters. Fluoride content also presented a strong positive correlation with well depth (R^2=0.63), implying more involvement of geologic processes than anthropogenic activities in raising the water fluoride content in the area. Tropical weather in the study area and slow groundwater flow may cause longer water-rock interaction time and increase the susceptibility of well waters for having a high fluoride content.

Keyword: Dental fluorosis, Well depth, Ghir, Fars, Iran, Arc GIS

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FLUORIDE CONCENTRATION LEVEL OF THE PERSIAN GULF SHORE IN BUSHEHR, IRAN

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Abstract:
Waterbody contamination can originate from different natural processes, human disposal practices, and industrial activities. Fluoride (F) in the environment is of special concern and its excessive intake can cause a wide range of adverse health effects. In this study, 48 samples were collected from 8 sampling points (6 samples for every point) in the coastal area of the Persian Gulf in Bushehr shore during the summer of 2013.
The mean F level of the all Bushehr shore samples was 2.63 mg/l with a range from 2.28 to 2.91 mg/l.

Keywords: Fluoride, Persian Gulf, coastal area

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EVALUATION AND COMPARISON OF FLUORIDE LEVEL IN BOTTLED, DISTRIBUTION NETWORK AND DECENTRALIZED MUNICIPAL DESALINATION PLANT WATER IN BUSHEHR IRAN

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Abstract:
In this study, the fluoride (F) concentration of the distribution network water supply, 10 water decentralized municipal desalination plants, and 10 most available brands of bottled drinking water in Bushehr, Iran were determined and compared. The mean F content of distribution network, decentralized municipal desalination plant and bottled water were 0.47, 0.16 and 0.18 mg/L; with a range of 0.42 to 0.59, 0.07 to 0.26 and 0.00 to 0.31 mg/L; respectively.

Keywords: Distribution network, decentralized municipal desalination plant, Bottled water

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LEVEL OF FLUORIDE CONTENT IN MEDICINAL PLANTS COMMONLY CONSUMED IN IRAN

Noushin Rastkari[a*], Reza Ahmadkhaniha[b], Nasrin Samadi[c]

Abstract

Herbal teas are sold for medicinal purposes, as well as a beverage. Herbal teas may consist of many ingredients, mainly botanical ingredients, and therefore are susceptible to contamination from the environment and manufacturing processes, which can interfere with their medicinal purposes. This research investigated the contamination of herbal teas by fluoride. The amount of fluoride in infusions of medicinal plants including Zataria, Matricaria chamomile, Borago, Spearmint, Roselle, Cinnamon, Ginger and Fennel was determined by potentiometric method using the fluoride ion selective electrode. The results demonstrated that the content of fluoride in herbal infusions ranged from n.d to 0.31 µg/ml (n.d. to 0.3 ppm) after 5, 10 and 15 min of brewing. The highest level was obtained for the infusion of Zataria and the lowest one was found in the extract of Fennel. It is found that brewing time (5, 10 and 15 min) increases the fluoride content in some cases. The survey results showed that fluoride levels in the samples is low and there is no risk of fluorosis

Key Words: Medicinal plants; fluoride; herbal infusion.

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ASSESSMENT OF FLUORIDE LEVEL IN DRINKING WATER RESOURCES OF BABOL RURAL REGIONS

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Abstract

Background & Objective: Fluoride is wide-spread in nature such as in soil and water. This element is also present in animal and plant tissue. Fluorine is one of the 14 elements essential for animal life in all tissues and body fluids of humans and animals and is present in measurable amounts. Drinking water with a fluoride content of one milligram per liter (1mg/L) is effective in reducing caries but sometimes a higher content with 1.5–2 mg/l can be cause mottled enamel. As the majority of the intake of fluoride into the body is from drinking water, the determination of water fluoride levels is important. The aim of this ecologic study was to determine of fluoride level in drinking water resources of the Babol rural regions in the northern part of Iran.

Materials & Methods: This study was conducted in 2012 on the drinking water sources in 47 villages of Babol, Iran. The samples were collected in two periods of the year to include seasons of both low and high rainfall. For the study, we sampled one liter of water in a plastic container from the total samples for all of the seasons and tested it with the standard SPADS method with a DR2500 spectrophotometer. The fluoride concentration in the samples was determined in mg/L and compared with the standard level (1–1.5mg/L). The data was analyzed using the SPSS software.

Results: The results showed that for one village source of drinking water the concentration of fluoride was greater than 1 mg/L at two periods of the year (2.1%). In seasons of low rainfall, 87.6% of the drinking water sources in the villages had a fluoride concentration lower than 0.5 mg/L and 10.6 % had a level of 0.5–0.7 mg/L. In seasons of high rainfall, 91.6% of the drinking water sources in the villages had a fluoride concentration lower than 0.5 mg/L and 6.3 % had a level of 0.5–0.7 mg/L.

Discussion & Conclusion: According to our results, the fluoride levels in the drinking resources of the Babol rural regions were lower than the global standards and, given the amount of tooth decay in northern Iran, it is necessary to add fluoride to the food chain of the population in the region. It will be beneficial for vulnerable groups, such as primary school students, to have fluoride applied through a mouthwash. The addition of fluoride through drinking water is the best method however and is recommended in these regions as a supplement.

Key words: Fluoride, Drinking water resources, Babol rural regions.

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STUDY OF CO- EXISTENCE OF FLUORIDE AND OTHER MINERAL CONTAMINANTS IN DRINKING WATER (A REVIEW STUDY)

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Abstract

The existence of fluoride and other mineral contaminants in ground waters has been widely reported. Their main source is from volcaniclastic material present in loess and alluvium deposits. A secondary source is Fe^{-}, Mn^{-}, Al^{-} oxides/hydroxides, and clay minerals that adsorb fluoride and other mineral contaminants. High evaporation rates, especial in arid and semiarid climates, generate saline ground waters and alkaline pH, releasing fluoride and others ions from both the primary and the secondary material sources, resulting in water rich in fluoride and other mineral contamination. Because of their health impacts, they should be analyzed and evaluated simultaneously. The present study, a review research, was carried out in 2013 and with the purpose of investigating the co-existence of fluoride and other mineral contaminations in the groundwater in arid and semiarid climates. We found high fluoride concentrations often show a direct relationship with high concentrations of other mineral contaminants. Enrichment of fluoride is generally associated with high Cl, Br, AS, and V concentrations. We concluded that more studies are require addressing the behavior of these contaminants, their health impact in arid and semiarid climates and with an alkaline pH. It is also is necessary to develop technologies and methods that are effective for simultaneously monitoring and removing these contaminants from drinking water.

Key words: Arid and semiarid climates; Drinking water; Fluoride; mineral contaminants

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FLUORIDE IN DRINKING WATER OF WEST AZERBAIJAN PROVINCE, IRAN

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Concern about adverse effects of fluoride in drinking water even in low concentration is growing day by day. As a result, responsibility of water supply organizations in providing safe water gets harder. Provision on quality of drinking water in Iran is the duty of ministry of Health.

Present study aimed to provide feature of exposure to fluoride via drinking water in West Azerbaijan (WA) province's counties including Sardasht, Mahabad, Tekab, Naghadeh, Chaypare, Khoy, Miyandoab, Oshnaviyyeh, Salmas, Bookan, Piranshahr, Shot and Poldasht (except Urmia) during 2011-2012. WA is in the northwest of Iran and covers an area of 39,487 km², or 43,660 km² including Lake Urmia. Population of province is more than 3,015,361. Required data (355 water analysis results) were gathered from the Health Center of WA province and analyzed by SPSS 16 and were compared with standards.

Fluoride concentration ranged from zero to 3.45 mg/L with a mean concentration equal to 0.32 ± 0.37 mg/L. According to the figure, high concentration of fluoride are observed in Makoo and Shot. There was significant positive correlation between fluoride concentration and EC, hardness, alkalinity, Ca, Mg, Na, K, SO4 of analyzed water samples.

Key words: Drinking water, Fluoride, West Azerbaijan, Iran

KNOWLEDGE, ATTITUDE, AND SELF-REPORTED PRACTICE OF FLUORIDE AMONG DENTISTS

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To assess dentist's knowledge, attitude, and self-reported practice toward preventive effects of fluoride we conducted a questionnaire survey at the nationwide annual dental congress in 2010 in Tehran, Iran. Dentists' level of knowledge was assessed based on their responses to statements on preventive effect of fluoride on a Lickert scale. Dentist's attitude toward preventive effects of fluoride for patient under 12-years-old and adults was evaluated. In addition, self-reported practice for two paper-patients (child and adult with high risk of dental caries) was assessed. 347 dentists, 232 (73.4%) male and 84 (26.6%) female responded. The majority agreed with adding fluoride to water (88.28%) followed by 69.4% for salt or milk fluoridation. However, 85.3% agreed that use of home fluoride in the form of fluoridated toothpaste is useful followed by 78.7% for fluoride rinse. 87.6% agreed that the professional application of fluoride as varnish, foam or gel is useful. In this study younger dentists were more knowledgeable than the others. The self-reported practice of fluoride application and lesion management on the two paper patients were rather similar with a more positive attitude towards using fluoride products for child patients. The majority of dentists (67.4%) reported they did not have access to clear guidelines for fluoride application. The younger and more recent graduates had a better level of practice compared to the others ($p<0.05$). Dentists' self-reported practice regarding application of fluoride products was weak in spite of having good level of knowledge.

Keyword: Fluoride, professional, oral health

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THE STUDY OF FLUORIDE CONCENTRATION IN SEPIDAN DRINKING WATER AND ITS RELATION TO DMFT INDEX IN 15-18 YEAR-OLD STUDENTS

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Abstract

Background and Aim: Fluoride in drinking water is one of the elements that can increase the tooth resistance toward the decay. Increasing this substance causes an increase in dental fluorosis and its deficiency may increase the decay. The DMFT index is the most important criterion for the quantitative assessing of dental caries which includes: number of permanent teeth (T), decayed (D), missing due to caries (M) or filled due to caries (F). The aim of this study was to determine DMFT in 18-15 year old students in Sepidan and its relation to fluoride in drinking water in 2012.

Material and Methods: This study was a cross-sectional analysis and the method used to collect data was based on observation, examination, and random sampling. The amount of fluoride in drinking water was supplied from the health center was measured. Examination of the teeth was done in the urban schools of Ardakan and rural schools of Khalili. A sample of the student population, with 95% confidence level and a standard deviation based on previous studies (2.5), was studied consisting of 186 male and female students.

Results: The mean fluoride concentration in drinking water was 0.24 mg/l. In this study, total DMFT index was 9.48. Highest mean DMFT was 10.05 in the 15-year-olds and the lowest index was 8.4 in the 18-year-olds. DMFT rates are slightly higher in boys than in girls, 9.61 and 9.38 respectively. Minimum and maximum amount of index was 7.35 (pre-university) and 10.34(secondary grade) respectively.

Discussion and conclusion: Given that the concentration of fluoride in drinking water in Sepidan is less than the amount recommended by the World Health Organization (0.5-1.5mg/l) and DMFT index in the city is very high compared with other regions, fluoridation of drinking water in Sepidan is recommended as one of the best options for reducing dental caries and diseases. In addition to the use of fluoride in drinking water, and fluoride intake from other fluoride topical sources, such as gel, mouthwash, toothpaste, is recommended.

Keywords: Fluoride, DMFT, drinking water.

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FLUORIDE REMOVAL FROM AQUEOUS SOLUTIONS USING SHRIMP SHELL AS A COST BENEFIT BIOSORBENT

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Abstract:
Adsorption is considered to be the most promising treatment technology for fluoride (F) removal from aqueous solutions. The purpose of this study was to determine the efficiency of shrimp shell in the removal of F from aqueous solutions.
Adsorption was studied in batch experiments at room temperature and the effects of experimental parameters such as adsorbent dose (0.8 – 64 g/l), contact time (5 -120 min) and initial F concentration (2 -8 mg/l) were studied.
The highest removal adsorption was at 18 g/l adsorbent, 60 min contact time and initial F concentration at 3 mg/l (98.5% F removal). It was concluded that the shrimp shell can be used as a successful and environmental friendly F adsorbent from wastewater effluents.

Keywords: Adsorption, shrimp shell, Fluoride

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FLUORIDE BIOSORPTION FROM AQUEOUS SOLUTIONS BY SARGASSUMHYSTRIX ALGAE

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Abstract:
Adsorption is considered to be the most promising treatment technology for fluoride (F) removal from aqueous solutions. The aim of this study was to determine the efficiency of *Sargassum hystrix* algae in the removal of F from aqueous solutions.
Adsorption was studied in the batch experiments at room temperature and the effects of experimental parameters such as adsorbent dose (0.8 – 64 g/l), contact time (5 – 120 min) and initial F concentration (2 – 8 mg/l) were examined.
The highest removal adsorption was at 40 g/l adsorbent, 60 min contact time and initial F concentration at 5 mg/l (100% F removal). It was concluded that the *Sargassum hystrix* can be used as a successful and environmental friendly F adsorbent from wastewater effluents.

Keywords: *Sargassum hystrix* algae, absorption, Fluoride

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FLUORIDE BIOSORPTION FROM AQUEOUS SOLUTIONS USING *MORINGA OLEIFERA* ASH

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Abstract:
Adsorption considered as the most promising treatment technologies for fluoride (F) removal from aqueous solutions. The aim of this study was to determine *Moringa oleifera* ash efficiency in removal of F from aqueous solutions.
Adsorption was studied in batch experiments at room temperature and the effects of experimental parameters such as adsorbent dose (0.8 – 64 g/l), contact time (5 -120 min) and initial F concentration (2 -8 mg/l) were studied.
The highest removal adsorption was at 64 g/l adsorbent, 10 min contact time and initial F concentration at 8 mg/l (81% F removal). The results showed that the *Moringa oleifera* ash can be used as an environmental friendly F adsorbent from wastewater effluents.

Keywords: Adsorption*, Moringa oleifera,* Fluoride

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FLUORIDE REMOVAL FROM AQUEOUS SOLUTIONS USING PADINA SANCTAE CRUCIS ALGAE AS BIOSORBENT

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Abstract:
Adsorption considered as the most promising treatment technology for fluoride (F) removal from aqueous solutions. The aim of this study was to determine *Padina sanctae crucis algae* efficiency in removal of F from aqueous solutions.
Adsorption was studied in the batch experiments at room temperature and the effects of experimental parameters such as adsorbent dose (0.8–64 g/l), contact time (5–120 min) and initial F concentration (2–8 mg/l) were studied.
The highest removal adsorption was at 48 g/l adsorbent, 5 min contact time and initial F concentration at 8 mg/l (97% F removal). It was concluded that the *Padina sanctae crucis* can be used as a successful and environmental friendly F adsorbent from wastewater effluents.
Keywords: *Padina sanctae crucis* algae, absorption, Fluoride

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REMOVAL OF FLUORIDE FROM URBANE DRINKING WATER BY BONE-CHAR

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Abstract:

The aim of this applied-analytical study was to investigate the feasibility of fluoride removal from drinking water using a batch reactor with two types of bone-char (white and black). The variables under study were pH, reaction time, adsorbent doses, fluoride concentrations, reaction kinetics, and bone char characteristics. A sample of urban drinking water was prepared containing 6-12 $mg.l^{-1}$ fluoride. Bone-char (B.C) was prepared in a laboratory electrical furnace at 450˚C and 900˚C for 4.5 and 8 h, respectively. The fluoride-containing water entered the batch reactor and the fluoride removal efficiency was studied in different cases of the variables pH (3-9), reaction time (0-60 min), and adsorbent doses (1-2 gr). Characteristics of B.C showed that the white bone-char (WBC) that prepared on 900˚C had low iodine number and BET surface area than black bone-char (BBC) that prepared on 450˚C. The main component of B.C was calcium phosphate ($Ca_5(PO_4)OH$). The findings indicated that fluoride removal efficiency was increased with decreasing pH, reaction time, and adsorbent doses. The best conditions for fluoride removal were found to be pH 7.5, reaction time 45 min, and adsorbent dose 2 g, and fluoride concentration 6 $mg.l^{-1}$. The adsorption of fluoride in two types of B.C was obtained from Langmuir isotherm.

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2- Keywords : Bone-char; Fluoride; Urbane drinking water

3- Water and health 2006; [04.1]: 2006: 139-147

PERFORMANCE COMPARISON OF ELECTROCOAGULATION PROCESS BY USING ALUMINUM AND IRON ELECTRODES IN DEFLUORIDATION OF DRINKING WATER

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Abstract

The aim of the present study was to compare the performance of a bipolar electrocoagulation (EC) reactor using iron and aluminum electrodes for fluoride removal from aqueous environments. The experiment was performed under different conditions of voltage (0–40V), detention time (0–40 min), pH (3, 6, 9) and concentration of initial fluoride (5, 10, 20 mg/l). At the end of each run, the residual fluoride concentration in the sample was determined by the SPANDS method and measuring its absorbance at 495 nm. The maximum efficiency of the electrocoagulation was achieved at a voltage of 40 V and a detention time of 40 min regardless of electrode types. Under this condition, fluoride concentration was reduced 94% and 69.5% by aluminum and iron electrodes respectively. According to the results, the electrocoagulation process by aluminum electrodes could be considered as more efficient way of fluoride removal.

Keywords: Electrocoagulation, Fluoride, Aluminum, Iron.

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A COMPARATIVE STUDY FOR EXCESS FLUORIDE REMOVAL EFFICIENCY BY DIFFERENT PROCESSES IN DRINKING WATER SUPPLIES (A REVIEW STUDY)

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Abstract

An excessive fluoride concentration in the groundwater supplied for human consumption will lead to a high risk of skeletal fluorosis. Fluorosis is a disease caused by drinking water with fluoride concentrations higher than 1 mg/l for long periods. In recent years, many studies have been carried out to find cost-effective and practical solutions for the removal of excess fluoride from drinking water. So far, different methods such as adsorption, reverse osmosis, nanofiltration, electrodialysis, Donnan dialysis, activated carbon, aluminum sulphate, poly-aluminium chloride, tricalcium phosphate, silica gel, zeolites, bentonite, and some other low-cost materials have been used for defluoridation. The purpose of this study was to compare the efficiencies of these different processes for removing excess fluoride in drinking water supplies. Our study, a research review research, carried out in 2013, reviewed the various methods of removal taking account of the economic cost and both chemical and physical parameters, e.g., initial amount of fluoride, electrical conductivity, pH, temperature; flow rate, turbidity. Our results showed that the fluoride removal efficiency was influenced by a number of factors including initial amounts of fluoride, economic cost, flow rate, and the presence of other mineral contaminants. We concluded that a comparison of the efficiency of the different defluoridation methods was the best way to find cost-effective and practical solutions for the removal of excess fluoride from drinking water.

Key words: Comparison; Drinking water; Fluoride removal; Water treatment

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MODIFIED EGGSHELL POWDER AS AN ADSORBENT FOR REMOVAL OF FLUORIDE FROM AQUEOUS SOLUTION

Sara Sadat Hosseini, Amir Hossein Mahvi

Drinking water provides many elements for the human body. One of these elements in drinking water is fluoride which has adverse health impacts in high concentrations. In this regard, the concentration of fluoride must be kept below the drinking water standards. There are different processes for removal of fluoride from water such as: coagulation, precipitation, ion exchange and membrane processes.

In this study eggshells were washed with double distilled water followed by drying in a hot air oven at 110^0C for 12hrs. The dried eggshells were ground to a powder using a blender and sieved well with a mesh size of 125 μm. The eggshell powder was put in a solution of 0.6 M $Al_2(SO4)_3$ by mixing and stirring for 4 hours. Adsorption experiments were carried out for determination of pH, adsorbent dose variation, contact time and selection of the relevant isotherm. The influence of pH was considered (3–9), adsorbent dose (0.15–2 g /100 ml), particle size (125 μm), contact time (30, 60, 90, 120 and 180 min) and initial fluoride concentration (3, 5, 7, 10 and 20 mg/L) . The sample solutions were collected for analysis and the fluoride concentration was determined by using a spectrophotometer. Each experiment was carried out three times and the average values were recorded. The results indicate that eggshell powder can be used as an effective low-cost adsorbent to remove fluoride from aqueous solution and reduce the fluoride concentration of drinking water to the standard levels.

Keywords: eggshell , Fluoride removal, absorbent, isotherm

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THE SURVEY ON THE MODIFIED SORGHUM AND CANOLA PERFORMANCE AS NATURAL BIOSORBENTS IN BIOSORPTION OF FLUORIDE FROM WATER: ISOTHERMIC AND KINETIC MODELING

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Abstract:

The uptake of high concentration of fluoride from water can lead to adverse effects such as flourosis. Therefore, the aim of this study was the investigation of Sorghum and Canola performance as natural adsorbents in fluoride removal from water. The adsorbents were dried in 105 for 24h. Then they were sieved to 2mm. The effect of various parameters such as pH, adsorbent dose, contact time and the fluoride concentration was investigated on adsorbent performance. The fluoride concentration was measured by spectrophotometer in =570nm. The isotherms and kinetic of adsorption was determined.

The results indicated that the fluoride removal by both adsorbents increase by increasing of the contact time and adsorbent dose. However, the removal rate reached to constant amount after 90 and 60 min with 8 g/l of Canola and sorghum dose, respectively. Optimum pH was 5 for fluoride removal. The adsorption data was followed the Langmuir No. 2 and pseudo-second-order kinetic. The studied adsorbents have good ability for fluoride removal and can be used as effective and low-cost adsorbents to treat the industrial effluents.

Keywords: Adsorption Canola, Sorghum, Fluoride, Water Treatment, Defluoridation

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INVESTIGATION OF MODIFIED MONTMORILLONITE BY POLYETHYLENEIMINE TO FLUORIDE REMOVAL FROM AQUATIC MEDIAS

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Abstract:
One of the major problems in ground water resources, known as the most important water resources for societies, is fluoride pollution. The potable water of over 200 million of the world population, living in USA, Africa, and Asia, is polluted with unacceptable levels of fluoride. In the present work, fluoride removal from aqueous solutions by natural montmorillonite, and modified montmorillonite using polyethyleneimine in a batch reactor was studied by applying Design Expert 7.0.0 Software, and performing experimental procedures with the analysis of four variables, namely, detention time, pH, initial fluoride concentration, and the adsorbent concentration on fluoride ion were conducted. It is noteworthy that all the chemical experiments conducted in this study were in accordance with "Standard Methods for Water and Wastewater Experiments". Also, XRD and SEM analysis were performed so as to determine the characteristics of the adsorbent. Based on the results, the fluoride removal efficiencies by applying natural montmorillonite (R^2= 0.969) and modified montmorillonite (R^2= 0.986) were respectively 71.5% and 80.66%. The data analysis was in accordance with polynomial model.

Key words: fluoride, montmorillonite, CCD, water

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OPTIMIZATION OF FLUORIDE ADSORPTION ONTO CARBONIZED PALM LEAVES BY EXPERIMENTAL DESIGN

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Abstract:
A study on the adsorption of fluoride onto Carbonized Palm Leaves (CPL) was conducted and the process parameters were optimized by using response surface methodology (RSM). Raw palm leaves were carbonized in the oven (250 ^{o}C) for 3 hours. In order to determine the effects of process parameters, initial solution pH (3-11), adsorbent dose (1-5 g/L), initial fluoride dose (2-14 mg/L) and time (0-240 min) on fluoride (F^{-}) uptake from aqueous solution, a five-level, four-factor, central composite design was employed. Analysis of the experimental data obtained from 28 batch runs showed that the maximum percentage for fluoride removal was 73.4% when pH, adsorbent dose, initial fluoride dose, and time were 9, 4 g/L, 5mg/L and 180 min, respectively. A quadratic kinetic model expressed well the fluoride adsorption onto the carbonized palm leaves. The most significant parameters were time ($P<0.014$) and fluoride dose ($P<0.047$), respectively.

Keywords: Adsorption; Carbonized Palm Leave; Fluoride; RSM.

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ADSORPTIVE REMOVAL OF FLUORIDE FROM AQUEOUS SOLUTION OVER MODIFIED PALM LEAVES: AN OPTIMIZATION STUDY

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Abstract:
This paper investigates, through response surface methodology (RSM), the optimization of the efficiency of the fluoride removal process by adsorption of fluoride from aqueous solution onto Modified Palm Leaves (MPL) using 0.7 M sodium bicarbonate. The parameters were initial solution pH (3–11), adsorbent dose (1–5 g/L), initial fluoride dose (2–14 mg/L) and time (0–240 min). A five-level, four- factor, central composite rotatable design (CCRD) was employed in 28 batch runs. Statistical testing by Analysis of Variance (ANOVA) and linear regression showed the most significant factor was fluoride concentration ($p<0.0001$). The removal efficiency of fluoride was optimized, with a maximum fluoride removal percentage of 50.8%, when pH=7, adsorbent dose=3g/L, initial fluoride dose=2 mg/L, and time=120 min.

Keywords: Adsorption; Fluoride removal by adsorption; Modified Palm Leaves; Response surface methodology.

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REMOVAL OF FLUORIDE FROM AQUEOUS SOLUTION BY USING RESPONSE SURFACE METHODOLOGY

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Abstract:
The presence of some fluoride in drinking water is useful for teeth and bones but excessive amounts may cause disease with bone abnormalities and skeletal fluorosis. A variety of technologies are available for fluoride removal and adsorption is a commonly used technique. This study was conducted on the adsorption of fluoride onto Raw Palm Leaves (RPL) using response surface methodology (RSM) for optimization. Parameters were initial solution pH (3–11), adsorbent dose (1–5 g/L), initial fluoride dose (2–14 mg/L) and time (0–240 min). A five-level, four-factor, central composite design was employed in 28 batch runs. The maximum fluoride removal percentage was 57.4% when pH, adsorbent dose, initial fluoride dose and time were 7, 3 g/L, 2 mg/L and 120 min, respectively. Linear mathematical analysis of the experimental data showed that fluoride dose ($p<0.0001$) and time ($p<0.039$) were the most significant factors.

Keywords: Adsorption of fluoride; Fluoride removal by adsorption; Palm leaves; Response surface methodology.

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INVESTIGATION OF Fe_3O_4 NANOPARTICLES COATED ON POWDER ACTIVATED CARBON EFFICIENT IN THE REMOVAL FLUORIDE FROM AQUEOUS SOLUTION

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Abstract:
Fluoride contamination of drinking water has been recognized as a major worldwide problem and is a serious threat to human health. It is, therefore, essential to remove fluoride from drinking water, by appropriate water treatment processes, so that its concentration is not above an acceptable level. The World Health Organization (WHO) regulations specify that the maximum acceptable concentration of fluoride ions in drinking water is 1.5 ppm. Various treatment technologies have been used for removing fluoride from aqueous solutions. The aim of this study was to determine the performance as an absorbent of Fe_3O_4-activated carbon magnetic nanoparticles (AC-Fe_3O_4 MNPs) synthesized from powder activated carbon (PAC) and magnetic nanoparticles of iron (III) oxide. The characteristics of the adsorbent were evaluated by SEM, TEM, XRD and BET. In this study, batch experiments were performed to investigate adsorption isotherms and the effects of experimental parameters such as contact time (5–180 min), pH (3–11), adsorbent dose (0.2–0.8 g/L) and various concentrations of fluoride (2–10 mg/L). The optimum fluoride removal was observed at a pH value of 5. This study found that the maximum absorption was rapid in the first 40 min and at 90 min the absorbance increased at a slower pace. We found that 94% of the fluoride ions could be extracted from water with a fluoride concentration of 5 mg/L under optimal conditions with a nanosorbents dosage of activated carbon adsorbent of 0.5 g/L, a contact time of 40 min, and a pH of 5. The data obtained from the experiments fitted the Langmuir model. The synthesized sorbent was successfully applied for treating a water sample.

Keywords: Activated carbon adsorbent; Adsorption of fluoride with nanoparticles; Defluoridation of water; Fe_3O_4-activated carbon magnetic nanoparticles.

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REMOVAL OF FLUORIDE FROM AQUEOUS SOLUTION BY ACID-TREATED CLINOPTILOLITE

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Abstract:
Fluoride contamination in drinking water due to natural and anthropogenic activities has been identified as a serious threat to human health. Among several treatment methods applied for fluoride removal, adsorption process has been investigated widely and offers satisfactory results especially with mineral-based and surface modified adsorbent. The present study describes the removal of fluoride from aqueous solution by using clinoptilolite, a low cost adsorbent when treated with acid. Adsorption isotherms and kinetics were also investigated. The characteristics of the adsorbent were evaluated by scanning electron microscopy (SEM), X-ray diffraction (XRD) and BET. The adsorption of fluoride on acid-treated clinoptilolite was studied as a function of contact time, pH, initial concentration, and adsorption dose. The experiments demonstrated that the maximum fluoride removal was obtained at a pH of 3 and it took 2 hr to attain equilibrium. The kinetics data fitted a pseudo-second-order model. The adsorption data was better described by the Freundlich isotherm model than the Langmuir isotherm model. Based on this study's result, acid-treated clinoptilolite is an affordable and a promising option for the removal of fluoride from aqueous solution.

Keywords: Fluoride removal with clinoptilolite; Acid-treated clinoptilolite; Adsorption of fluoride; Kinetics of fluoride adsorption.

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RT-PCR AMPLIFICATION OF COAT PROTEIN GENE OF *CITRUS PSOROSIS VIRUS* IN IRAN

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Abstract:
Citrus psorosis virus (CPV), the type species of the genus *Ophiovirus* of the family *Ophioviridae*, is the causal agent of Psorosis, one of the most widespread and economically important diseases of citrus. CPV-infected trees show oak leaf and flecking patterns on leaves and bark scaling. CPV virions are spiral filaments containing a tripartite single stranded RNA genome of negative polarity and a 48 KD coat protein encoded by RNA3. An isolate of CPV recovered from northern Iran was graft-transmitted from an infected sweet orange [*citrus sinensis* (L.) Osb.] tree to seedlings of a number of citrus species and cultivars. Symptoms appeared 6-8 weeks post inoculation. Total RNA was subsequently extracted by our optimized method from mature leaf tissues of infected seedlings as well as seed-grown healthy plants as a negative control. Five primer pairs complementary to various regions of CPV coat protein (CPV-CP) gene were applied for RT-PCR amplification of partial and complete sequences of different lengths. Furthermore, the ICRSV primer pair reported for RT-PCR amplification of coat protein gene of ICRSV (*Indian citrus ringspot virus*) was also included in the trials. Complementary DNA (cDNA), synthesized on 100 ng of total RNA using *M-Mulv* reverse transcriptase in the presence of forward and reverse primers, was amplified in PCR reaction by *taq* DNA polymerase. Specific fragments of the expected size appeared with all five primer pairs of CPV-CP, whereas no amplification occurred when the ICRSV primer pair was used. Nevertheless, four primer pairs were surprisingly amplified by those fragments in the healthy sample as well; including a 600 bp fragment amplified by the CPV1 and CPV2 primer pairs, which was verified by sequencing and homology alignment as a part of the rRNA molecule of citrus host origin. The amplification product with CRSVIF-2 and CRSVIR-2 primer pair was a fragment with an estimated size of 1200 bp, which was not present in the healthy sample. However, this fragment had a low concentration and was accompanied by nonspecific bands of different sizes. Gel purification and sequencing of the fragment is being attempted.

Key words: *Citrus psorosis virus*; CPV; Citrus; RT-PCR; Specific primer pairs.

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OPTIMIZATION OF RNA EXTRACTION METHOD FOR MOLECULAR STUDIES OF *CITRUS TRISTEZA VIRUS* AND *CITRUS PSOROSIS VIRUS* IN CITRUS

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Abstract:
Psorosis, caused by *Citrus psorosis virus* (CPV), is one of most prevailing diseases of citrus worldwide. CPV has three negative sense single stranded RNA genome, encapsidated into a 48 KD coat protein encoded by RNA3. Presence of the CPV was initially recorded on 1992 in northern Iran. Its biological properties were studied following graft-inoculation onto some citrus species and cultivars as indicator plants. Because of the low virion titer in citrus hosts, CPV RNA extraction is difficult and no data has been published about its molecular properties in Iran. We report here an attempt to extract high yield and quality of total RNA from CPV-infected citrus leaves. Three commonly used methods including TRizol, Derrick *et al*., (1998) and Hung *et al*., (2000) were also included for total RNA extraction and evaluation of the potential of our Optimized method. Briefly, in the optimized method, 1 g of mature leaf tissues of a CPV graft-inoculated Yuzu (*C. junos* Sieb. Ex Tan.) tree was powdered in liquid nitrogen and homogenized in 10 ml of extraction buffer [50 mM Tris-Cl pH 8.0, 2% SDS, 0.5% 2-Mercaptoethanol, 10% Sarkosyl]. Following incubation at 55°C for 1h, acidic phenol was added, mixed and centrifuged at 4000 rpm for 5 min. Ten percent CTAB (Cetyl trimethylammonium bromide) and 5 M NaCl were added to supernatant and incubation continued at 65°C for 10 min to remove plant polyphenolic compounds and polysaccharides. After twice phenol:chloroform (1:1) extraction, the upper phase was aspirated and precipitated with isopropanol at 4°C and resuspended in 100 µl of *RNase*-free ddH_2O. Quantity and quality assessment of total RNA extracted by all four methods, carried out by spectrophotometry and agarose gel electrophoresis, showed approximately two-fold concentration of RNA obtained by optimized method and no DNA contamination as well. Furthermore, when total RNA extracted by the optimized method was used for RT-PCR amplification of CPV coat protein gene, a 1200 bp fragment of expected size was appeared in agarose gel whereas no amplification or low intensity of amplified fragment was observed with RNA prepared by other three methods.

Key words: Citrus; *Citrus psorosis virus* (CPV); RNA extraction; The optimized method.

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EVALUATION OF FORCES APPLIED ON SELECTIVE JOINTS AND MUSCLES OF DRIVERS DURING CLUTCHING OF MF285 AND MF399 TRACTORS

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Abstract

In this paper the forces imposed on three muscles, Gastrocnemius muscle, Trapezius muscle and Quadratus lumborum muscle, of the tractor driver during clutching were studied. Thirty subjects were studied and the research was conducted on MF285 and MF399 tractors. The clutching forces for these tractors were measured as 340 N and 290 N, respectively. The angle of knee location in these two tractors at 5 percent level was significantly different. The decrease of pain threshold after 30 seconds and 60 seconds clutching and 60 seconds rest after clutching in MF285 tractor in all three muscles were more than MF399 tractor. It is suggested that in order to decrease the imposed force of clutching for the MF285 tractor some modifications are required. In this regard, the force transfer joint between the pedal and the disc in the mechanism of clutching could be made of cast iron.

Keywords: ergonomic, tractor, operator, muscle, algometer

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STUDY OF FLUORIDE HEALTH EFFECTS IN DRINKING WATER SUPPLIES IN SARAYAN CITY IN IRAN

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Abstract:

Fluoride is an essential microelement for human health with the intake for humans coming from different sources. One of these resources is drinking water. Exposure to levels of fluoride higher and lower than the optimal levels can lead to fluorosis and dental decay respectively. The minimum and maximum allowable levels of this microelement in water for a region also depends on the average annual maximum daily air temperature. According to the Iranian national standard the optimal amount is 0.5 to 1.5 mg/l. Because of the effects of fluoride on health, we evaluated the amount of fluoride in the drinking water supplies in Sarayn, a city in Iran.

Our study was a cross-sectional study carried out, during 2010 to 2012, on the seven ring aqueducts, on two deep and handheld wells, and on basic information collected from the water and waste water company and the clinical health network in Sarayan city. In this study, the fluoride concentration was detected by using an ion-selective electrode. To compare the fluoride concentrations with the Iranian national standard in drinking water supplies, was used the ANOVA and SPSS ware.

The results showed that the minimum (0.07 mg/l) and maximum (0.48 mg/l) amounts of fluoride were present in Charmeh and Baghdadh villages respectively. Fluoride concentrations in drinking water supplies did not differ significantly between various years ($p<0.05$). The fluoride content in the drinking water in Sarayan city was lower than ranges recommended by the Iranian standard and the World Health Organization. Also, based on information gathered from the clinical health network, the percent of caries free children aged 6 to 7 years in Sarayan was lower than those in *Global Goals for Oral Health 2010*.

We considered that the low levels of fluoride in the drinking water in Sarayan increased the risk of tooth decay and that further studies looking at increasing the intake of fluoride from water or other sources were appropriate.

Key words: Effect health; Fluoride; Sarayan; Water drinking

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INVESTIGATION OF RELATIONSHIP BETWEEN BIRTH WEIGHT AND LENGTH WITH MOTHERS FLUORIDE EXPOSURE IN DRINKING WATER IN WEST AZERBAIJAN PROVINCE, IRAN

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Abstract:

Birth weight and length have correlated positively with the adult body mass index (BMI). Mothers fluoride exposure in drinking water may be affect the birth weight and length. In this cross-sectional study, two groups of babies, which their mothers were exposed to high (3.94 mg/l) and low (0.25 mg/l) fluoride of drinking water, were perused. According to the result, the average birth weight and length were 3200 ± 404 gram and 49.04 ± 2.76 centimeter; and 3167 ± 494 gram and 50.57 ± 2.71 centimeter in high and low fluoride areas, respectively. Although there was no statistically significant difference between the means birth weight in two areas, the means birth length possessed significant difference in two areas ($P<0.001$), owning the different mothers fluoride exposure in drinking water. The results demonstrated that the more the fluoride exposure in drinking water, the less the mean birth length. Thus, excessive levels of fluoride in drinking water should be removed by appropriate treatment methods.

Keywords: Birth Weight and length; Fluoride in drinking water; West Azerbaijan.

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INVESTIGATION OF HYPERTENSION IN PEOPLE EXPOSED TO HIGH AND LOW FLUORIDE IN DRINKING WATER IN WEST AZERBAIJAN PROVINCE, IRAN

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Abstract:
The blood pressure is a major public health challenge in Iran. Thus detection of hypertension and blood pressure control are critically important for reducing the risk of heart attacks and strokes. Fluoride could be one of the risk factors for incidence hypertension. In this cross-sectional study the effects of high and low fluoride exposure via drinking water on prevalence of hypertension was investigated in two area in west Azerbaijan province. Two study groups were seventy people (22 males/ 48 females) in high fluoride area (3.94 mg F/l) and one hundred forty-two people (50 males/92 females) in low fluoride area (0.25 mg F/l). The result is shown 3.1% and 2.7% of people were suffering of hypertension in group with high and low fluoride exposure, respectively. Also based on chi-square statistical analysis there was not significant differences between hypertension and Fluoride in drinking water ($P>0.05$). In conclusion it is evident Fluoride has not direct effect on hypertension but suggested more study in this field.

Key word: Drinking water; Fluoride; Hypertension; West Azerbaijan.

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