APPENDIX D.

FAN's response to EPA's criticisms of submitted health studies.
(Submitted March 23, 2004)

In this section we examine EPA's responses to several of the key studies on health effects. These include:

1) Clinical bone trials
2) Bone fracture study of Li et al. (2001)
3) Bone fracture study of Alarcon-Herrera et al. (2001)
4) Cortical bone defects found in the Schlesinger et al. study (1956)
5) The pineal gland (luke, 1997, 2001)
7) The thyroid gland (Bachinskii et al, 1985).
8) G-proteins
9) Reproductive effects (Freni, 1994 and other studies)
10) The central nervous system.
10) Osteosarcoma and chromosomal damage.

Note: The EPA comments we cite come from the following two documents:

> November 18, 2003. A Preliminary Evaluation of Articles Related to Fluoride Cited by the Fluoride Action Network FAN) as Objections to the Sulfuryl Fluoride Pesticide Tolerance Rule. Docket No. OPP-2003-0373-0003.
> Comments Prepared by:
> Karl Baetcke, Ph.D., OPP/HED
> Jerome Blondell, Ph.D., OPP/HED
> William Burnam, Ph.D., OPP/HED
> Vicki L Dellarco, Ph.D., OPP/HED
> Joyce Donohue, Ph.D., OW/HECD
> Richard Hill, M.D., OSPC
>
> January 16, 2004. RESPONSE TO PUBLIC COMMENTS CONCERNING THE USE OF SULFURYL FLUORIDE AS A POST-HARVEST FUMIGANT. Docket No. OPP-2003-0373-0006. No authors listed.
> .

1 BONE. CLINICAL TRIALS. Surprisingly, Baetcke et al.'s weakest analysis was their very selective response to studies on bone. This is surprising because this is the only end point that EPA admits, albeit in the extreme case of "crippling skeletal fluorosis". This is most apparent in their cavalier dismissal of the clinical trials where patients with osteoporois have been treated with fluoride (on average 26 mg per day) for 1 -4 years in order to increase their bone mineral density. The hope of those conducting these trials was that this treatment would reduce the petient's risk of bone fracture, especially of the hip. Unfortunately, these trials actually resulted in an increase in hip fractures.

Here are the EPA's responses to three of the trials we listed:

1. (ref 4.) Bayley TA et al. (1990). Fluoride-induced factures: relation to osteogenic effect. J Bone Miner Res. Mar;5 Suppl 1:S217-22.

EPA RESPONSE:

> "An osteoporotic study using high doses of fluoride (60 mg/day) which are above the current EPA MCL ."

2. (ref 36.) Gutteridge DH et al. (2002). A randomized trial of sodium fluoride (60 mg) Estrogen in Postmenopausal Osteoporotic Vertebral Fractures: Increased Vertebral Fractures and Peripheral Bone Loss with Sodium Fluoride; Concurrent Estrogen Prevents Peripheral Loss, But Not Vertebral Fractures. Osteoporosis International. Vol. 13 No. 2: 158-170.

EPA RESPONSE:

> "An osteoporotic study using high doses of fluoride (60 mg/day) which are above current EPA MCL"

3. (ref 38). Hedlund LR, Gallagher JC (1989). Increased incidence of hip fracture in osteoporotic women treated with sodium fluoride. J Bone Miner Res Apr; 4(2):223-5.

EPA RESPONSE:

> "Increased incidence of hip fracture in osteoporotic women found at fluoride levels (50mg/day) higher than current OW MCL.."

In all three of these responses, the EPA reviewers have got the dose wrong. They have reported the dose as "fluoride", when in actuality the number they use is for "sodium fluoride". The correct fluoride dose actually used in the clinical trials was less than half of the numbers they cite. On average the daily dose used in clinical trials reporting increased bone fractures is 26 mg per day (mg/day of fluoride = mg /day of sodium fluoride x 19/44).

In all three of these responses, the EPA reviewers miss the point when they dismiss the significance of these findings because the daily dose is above the EPA's MCL of 8 mg per day. We made the point in our submission that these findings raise the question "that if relatively high doses over a short period of time make bones more brittle to fracture, what about lower doses over much longer periods of time?" In other words our concern is about <u>cumulative</u> dose (daily dose x number of days) not <u>daily</u> dose, i.e. not 26 mg per day for 1-4 years, but 8 mg per day for a lifetime.

A far more sophisticated analysis is demanded on this issue than the simplistic one of comparing 8 versus 26 mg per day. Moreover, it is essential that EPA apply a margin of safety to the doses producing fractures in these trials. This is particularly important considering the relatively short term nature of the trials.

2. BONE. Li et al. (2001)

In our submission we wrote that:

> Li et al. (2001) looked at hip fracture rates in elderly residents in six Chinese villages with different levels of fluoride in their well water. They determined a relative risk ratio for each of six villages taking the level of hip fractures in the village with 1 ppm as their reference. While they found little difference in the hip fracture rates in the villages less than 1 ppm, they found that the rates almost doubled when the levels of fluoride went above 1.5 ppm and tripled when they went over 4.5 ppm. This apparent dose response adds a great deal of weight to this ecological study. The doubling of the hip fracture rates above 1.5 ppm and the tripling of the hip fracture rates at levels over 4.5 ppm, puts into serious question the safety of the US EPA MCLG of 4 ppm.

The EPA claims that:

> "The Agency has found the results of the Li, et al. (2001) study cited by the Fluoride Action Network to be inconclusive due to inadequate exposure assessment, potential biases associated with misclassification of exposure, the failure to examine fractures other than hip separately, and limited statistical analysis found in this report."

In FAN's view this is a cavalier dismissal of a paper, which appeared in the prestigious ***Journal of Bone and Mineral Research***, and found a statistically non-significant doubling of hip fractures at 1.5 ppm (compared to a control village at 1 ppm) and a statistically significant tripling of hip fracture rates for villages with water greater than 4.3 ppm in their well water. Moreover, Baetcke et al fail to acknowledge that Sowers (1991) had found an increase in hip fracture in the US at 4 ppm, so the result did not come out of the blue. The EPA fails to acknowledge the key finding- namely the apparent dose response in the data. Again, the detailed nitpicking here is in sharp contrast to the EPA's blind acceptance of data collected over 60 years in Denmark, which they have accepted on the basis of one sentence from one pro-fluoridation scientist.

3. BONE. Alarcon-Herrera et al. (2001)

In our submission we wrote that:

> Alarcon-Herrera, et al. (2001) in a study conducted in Mexico found a linear correlation between the severity of dental fluorosis in both children and adults and the incidence of bone fracture.

The EPA's response to Alarcon-Herrera was as follows

> “The Alarcon-Herrera, et al. (2001) paper was also found to be inconclusive. The report shows some evidence of non-traumatic fractures in children associated with the two highest fluoride levels. But, the two highest levels occurred only in rural areas where children are likely to be more active out-of-doors where rough and tumble play might lead to fractures without any immediate cause being apparent. The authors do not mention this important confounder. It also appears likely that exposure to other sources of fluoride may have influenced the results of this study. In addition, mobility within the valley could have led to exposure misclassification. The occurrence of 7% fluorosis in the lowest exposure group strongly suggest this possibility. Therefore, it is not possible to determine, from this study, what the contribution of fluoride in drinking water is to increasing risk for nontraumatic fractures.
>
> The report was not inconclusive on the study's key finding: the authors found a linear correlation between the severity of dental fluorosis and the incidence of bone fractures in both children and adults. Here again, the EPA reviewers miss the point. The beauty of the Alarcon-Herrera study is that the biomarker the authors use is independent of the source of fluoride. The severity of dental fluorois is a well established biomarker for fluoride exposure in children. This study indicates that the more fluoride the children were exposed to, the greater the incidence of bone fracture. Thus the argument by the EPA that, " exposure to other sources of fluoride may have influenced the results of this study..." and "it is not possible to determine... what the contribution of fluoride in drinking water is to increasing risk for nontraumatic fractures" shows a serious misunderstanding of the key finding of this study. Based upon their comments here, it would appear that the EPA is asking for a return to an "ecological" study rather than one that relates to individual dose.”

The EPA's failure here is particularly regrettable, because one of the concerns that FAN has (and many others) is that the dental fluorosis incidence for children in the US in 1 ppm communities is about three times higher than that originally intended by the promoters of water fluoridation (Heller et al, 1997) indicating excess exposure to fluoride. Proponents of fluoridation always dismiss such findings because they claim that dental fluorosis is a "cosmetic" effect not a health effect. However, if we acknowledge the Alarcon-Herrera findings, then increased dental fluorosis figures may be a warning of increased bone fracture rates in children.

4. BONE. Schlesinger et al. (1956).

Schlesinger ER et al. (1956). Newburgh- Kingston caries-fluorine study XIII. Pediatric findings after ten years. Journal of the American Dental Association, 52.

In one of the early trials of fluoridation, Schlesinger and co-authors compared the health of the children in Newburgh, NY with children from the unfluoridated control city of Kingston, NY, over a ten year period from 1945-55. One of the surprising findings was a greater incidence of cortical bone defects in the children in fluoridated Newburgh which was statistically significant (cortical bone is the outside layer which is key for protection against fractures). This was not given much significance at the time but in the light of Alarcon-Herrera's findings above, it becomes extremely interesting. But not apparently so for the EPA. Here is what Baetcke et al wrote:

The EPA Response to this study:

> "Historical study that has been considered by many groups, and thus will not impact MCL. This study was considered by the 1993 NAS report."

It may not have interested the NRC in 1993, but surely after the publication of the Alarcon-Herrera study it should interest the newly convened NRC panel? Cortical bone is the outside layer of the bone which is key for protecting the appendicular skeleton against fractures. Could it not be that causing defects in this layer makes children's bones more prone to fracture? If it does, it certainly would impact the MCL since these defects are occuring at 1 ppm, which is four times lower than the MCL, even before applying a safety margin!

5. PINEAL GLAND. Luke (1997, 2001).

In FAN's submission we pointed out that:

> Fluoride accumulates in the pineal gland and may reduce melatonin production. The researcher Jennifer Luke discovered that the pineal gland is not protected by the blood brain barrier, has a high diffusion rate of blood and that it was also a calcifying tissue (it lays down the same crystals of calcium hydroxy apatite as are produced in the teeth and bones). Eleven corpses of elderly people were analyzed and it was determined that the levels of fluoride in the crystals in the pineal gland were extremely high (a mean of about 9000 ppm). This research was a PhD thesis sent to EPA and published in Caries Research (Luke 2001). The four step process from tryptophan (to melatonin) involves production of the neurotransmitter serotonin. It is conceivable that the production of this important substance is also lowered by the high concentration of fluoride - a well known enzyme inhibitor - in the pineal gland.

To these comments the EPA responded:

> "The effects of fluoride on the pineal gland have been reported only by one author in one study. The author states that the interpretation that depressed melatonin levels in the blood may hasten the onset of puberty is "conjectural". Because animal data on the effects of fluoride and the pineal gland comes from a single study with limited number of animals with only two dose levels, these

> findings should be confirmed by other laboratory studies. Also, the single report by the same author (J. Luke) on fluoride deposition in the aged human pineal gland from cadavers provides no data associating fluoride exposure with adverse effects in humans."

FAN emailed this response to Dr. jennifer Luke for her response to EPA's comments, she replied:

> Reading through your email again this evening, it would seem that, in replying to your comments, they have taken my reticence in the conclusions from my Ph D to heart. Rightly so, the work should be repeated although I have no doubts whatever that the result would be the same. The work was stringently carried out. I would like to point out that the artificial fluoridation of drinking water departs significantly from the normal situation as regards F-intake. This is especially serious in infants and young children. Where my study differs from other studies is that the animals received F from day 1 (in this way attempting to simulate the human situation where bottle fed infants often receive F-water to reconstitute their feeds immediately after birth). This is in contrast to other studies where animals receive the F-dose after weaning.
>
> High plasma F–levels neonatally and for the fist few months of life may alter pineal indole metabolism with affects on the hypothalamus/gonadal axis.
>
> Perhaps the most honest approach would be to make funds available for a research study in this area. (email, March 21, 2004)

Luke's second comment echoes the concerns of many independent scientists when they consider the significance of dosing newly born babies with formula made up with fluoridated water. With 1 ppm fluoride in the water these babies will be receiving doses of fluoride which are 100 to 200 times higher than the levels that they would be getting from mothers' milk which contains fluoride at the very low levels of 0.005 – 0.01 ppm (Institute of medicine, 1997). With water at the MCL (4ppm) these babies will be getting 400 to 800 times the natural level. It was concerns about what such levels of fluoride might do to the developing brain that was one of the key reasons offered by Dr. Arvid Carlsson (Nobel Laureate in Medicine) for opposing fluoridation in Sweden in the 1970's and more recently prompted this comment from Dr. Vyvyan Howard, an infant and fetal pathologist from the University of Liverpool:

> "Nature appears to have evolved a mechanism of minimizing the exposure of infants to fluoride. Human breast milk only contains between 5 and 10 ppb fluoride… chloride, a closely associated halogen ion that is essential for life, is present in breast milk at 360,000 parts per billion. There must be an evolutionary selection pressure operating for this selective exclusion of an otherwise highly diffusible ion."

At least the EPA has acknowledged the existence of Luke's work, which makes it the first agency in any fluoridated country to do so. However, the author's dismissal of the accumulation in the human pineal gland on the basis that there was "no data associating fluoride exposure with adverse effects" is as cavalier as it is premature, since no agency has actually looked for the effects that might be associated with this disturbing finding. It very much highlights the different attitudes in mainland European regulatory circles and those in America. In Europe the endorsement of the "precautionary principle" is almost instinctive, hence their reluctance to endorse fluoridation, genetic engineering and other American enthusiasms. It is ironic that at a time when many countries are considering banning certain organic chemicals (the POPs Treaty) simply because they accumulate in the environment, that we should liberally expose our population to more and more sources of this chemical even though we have known for years that it accumulates in our bones, and now has been shown to accumulate in the pineal gland. The prevailing attitude in America is that chemicals should have the same rights as people: they are assumed to be innocent until proven guilty. Such an attitude may serve the interest of chemical companies but it is not in the public interest, because finding such proof is a very lengthy business and during that time many millions may be irreversibly harmed. The Precautionary Principle suggests that a chemical should be assumed guilty unless evidence is produced to demonstrate its innocence.

We would further add that one of the first steps in risk assessment is hazard identification. It surprises us that the EPA is not indicating any steps to explore this hazard, before permitting even more fluoride to enter our food and accumulate in our pineal glands.

The EPA glosses over another finding made by Luke when she reviewed the literature on this matter. She noted a finding in the health study in the Newburgh-Kingston fluoridation trial (which was not thought significant at the time) that on average the girls in Newburgh started menstruating 5 months earlier than the non-fluoridated Kingston girls (Schlessinger et al, 1956). Thus one of the risks we may be taking by exposing our whole population to fluoride is interfering with delicate regulatory timing processes, from the onset of puberty to the aging process.

The EPA responded to this comment :

> “... the authors of the Newburgh-Kingston study concluded that "No differences of medical significance could be found between the two groups of children; thus further evidence was added to that already available on the safety of water fluoridation." The Newburgh studies (Ast and Chase, 1953; Ast, et al., 1956) were considered by the 1993 NAS review and thus do not provide new information on fluoride. Luke stated that fluoride may result in an early onset of puberty in treated gerbils but stressed that these findings were preliminary and this interpretation was conjectural. The Agency agrees with Luke that no firm conclusions should be drawn from this gerbil study. Furthermore, FDA did not

> observe an effect on puberty in their developmental and reproductive studies in rats (Collins, et al. 2001; Sprando 1997)."

The EPA's dismissal of the findings in the Newburgh-Kingston fluoridation trial of earlier menstruation (by 5 months) in the children in the fluoridated community, compared to the non-fluoridated community, is also disturbing. To argue, as the EPA does, that the authors of this study declared in 1956 that "No differences of medical significance could be found between the two groups of children" as a reason for ignoring it today in the light of Luke's findings is foolish. Similarly, the fact that this study may have appeared in the reference list of the NRC (1993) review of the MCL, should not be taken to mean that this particular finding was actually considered by the NRC panel in any detail, or with Luke's findings in mind (they were not published in 1993). Hopefully, the current NRC panel will take a second look at this finding.

Before giving too much credence to the EPA's comment that the "FDA did not observe an effect on puberty in their developmental and reproductive studies in rats (Collins, et al. 2001; Sprando 1997)" readers should be aware that Sprando and Collins in their studies have not been able to confirm many other findings reported by quite a number of researchers around the world (see discussion on reproductive effects below).

6. THYROID GLAND.

In FAN's submission we identified some of our concerns with fluoride's impact on the thyroid gland. Here are our concerns accurately paraphrased by the EPA:

> In the past sodium fluoride tablets have actually been given to patients to relieve the symptoms of hyperthyroidism (Galletti and Joyet, 1958). Independent observers have argued that if fluoride can lower the activity of the thyroid gland of someone suffering from an overactive thyroid gland, it might also reduce the activity of a normal thyroid gland and thus produce the symptoms of hypothyroidism, or it might further exacerbate the problems with those with existing hypothyroidism. Of particular concern, in this respect, is the fact that millions of people in the US are suffering from hypothyroidism. According to the DHHS the range of doses adults receive who live in optimally fluoridated areas is 1.6 to 6.6 mg/day (DHHS, 1991). This range overlaps the range of doses used in the Galletti and Joyet (1958) treatment regime for hyperthyroidism (2.3 - 4.5 mg per day).

We drew particular attention to a study by Bachinskii:

> Bachinskii et al. (1985) treated 123 people with elevated levels of fluoride(2.3 ppm) in their drinking water. He found that this treatment elevated TSH production, decreased T3 levels, and increased the uptake of radioactive iodide into the thyroid gland. Of the 123 people examined, 47 had normal thyroid function, 43 were hyperthyroid, and 33 were hypothyroid.

The EPA responded:

> "The Agency has reviewed the papers cited by the Fluoride Action Network that deal with the effects of fluoride on thyroid functioning. The papers submitted by FAN do not convince EPA that fluoride produces significant effects on the thyroid because of study design and report deficiencies."

This is a strange response because when we eventually found their response to the Bachinskii paper (in their tabled responses to all our references), we discovered that they weren't able to read it because it was published in Russian! Here is their response to the Bachinskii paper which appeared in Table 1 a (Baetcke et al, 2003):

Ref. 3 Bachinskii PP et al. (1985) Action of the body fluorine of healthy persons and thyroidopathy patients on the function of hypophyseal-thyroid system. Probl Endokrinol (Mosk) 31(6):25-9.

EPA response:

> "Russian paper; abstract is in English. Without a translation, can not discern from the numberical data the significance of the influence of fluoride levels on thyroid-pituitary function. It is also not known to what extent there may have been fluoride sources other than drinking water. Predates the 1993 NAS report, thus contains no new information."

We do not find their inability to get a Russian translation of this paper a satisfactory reason for dismissing this important work. Nor is their comment that because it predates the 1993 NAS (NRC) report "it contains no new information". This would only be helpful if the 1993 NRC panel had actually reviewed this study, but it did not. If neither the NRC nor the EPA were able to review this paper, how does the EPA know that "it contains no new information" ?

The way the EPA dismissed this paper is a clear example of their trying to dismiss our concerns, and the references that support them, in any way they can, whether their arguments make any sense or not. This kind of attitude betrays their agenda. They seem more anxious to protect DOW's petition, than they are to pursue the scientific evidence wherever it takes them.

Through the extensive network of scientists and citizens concerned about over-exposure to fluoride worldwide, FAN has been able to have the Bachinskii paper translated, it appears in Appendix E.

7. G-PROTEINS.

In FAN's submission we expressed our concern about fluoride's ability to switch on G-proteins:

> It is now well established in the biochemical literature that fluoride in the presence of trace amounts of aluminum is capable of switching on the G-protein signaling mechanism used for the transmission of signals which arrive at the outside of cells and result in changed activity inside the cell. These messengers include many water soluble hormones, some neurotransmitters, and some growth factors. It would appear that AlF4- can sit in the pocket on the G-protein that is normally occupied by the third phosphate of guanosine triphosphate (GTP). Normally the G-protein is in the "off" position when guanosine diphosphate (GDP) occupies the site; and in the "on" position when GTP occupies the site. However, when the site is occupied by GDP and AlF4-, it looks to the G-protein as if GTP is present, and is thus switched "on." The GDP (off) - GTP (on) switch is normally triggered when a messenger arrives at the receptor on the outside of the membrane. With AlF4- present the G-protein is switched on without the messenger. It is thus activated without the arrival of the normal messenger. The activated G-protein in turn activates the enzyme (adenyl cyclase) which converts ATP to cyclic AMP, which in turn excites a cascade mechanism resulting in changes inside the cell.

We further elaborated the fact that this could mean that fluoride could interfere with not just with one hormone but many hormones as well as other water soluble messengers, like growth factors. This is important because both DOW and the EPA have argued that there is no evidence that fluoride is an endocrine disruptor. We stated in our submission that:

> As this G-protein signal is a key step in the mechanism of action of many water soluble hormones, a number of neurotransmitters and growth factors, this interference by fluoride, in the presence of a trace amount of aluminum, is very worrying indeed. If one goes to the PubMed web and enters fluoride and G-proteins one gets about 800 hits. An important review of this issue and a good starting point for many of these references is provided by Strunecka and Patocka (1999). It is surprising to us that DOW is unaware of this serious biochemical role of fluoride.

The EPA responded to these comments:

> “The effects of Aluminum-fluoride complexes on G-protein and the enzymes associated with G-protein activation have only been demonstrated in vitro or when injected directly into the brain of laboratory animals. Thus, many significant questions still need to be addressed regarding biological availability and relative affinity for cellular and subcellular sites following human exposure (Baetcke, et al. 2003). The Agency has considered the new information on fluoride, and is not convinced that the data support the statement that fluoride is an endocrine disruptor. Dietary exposure to fluoride has not been shown conclusively to result in effects on reproduction, development or on hormones.”

In Table 1a, Baetcke et al. handle Strunecka and Patocka's important review as follows.

(Ref. 67) Strunecka A and Patocka J (1999). Pharmacological and toxicological effects of aluminofluoride complexes. Fluoride, 32, 230-242.

EPA RESPONSE:

> "Review on aluminofluoride complexes, no original data on fluoride."

The EPA also commented on the paper by Matsuo et al. (1998) who offered activation of G-proteins as the mechanism underpinning dental fluorosis.

(Ref.100) Matsuo S, Kiyomiya K, Kurrebe M. (1998). Mechanism of toxic action of fluoride in dental fluorosis: whether trimeric G proteins participate in the disturbance on intracellular transport of secretory ameloblast exposed to fluoride. Arch Toxicol 1998 Dec; 72(12); 798-806.

EPA RESPONSE:

> "Mechanism paper on dental fluorosis using doses higher than the current secondary MCL"

In a late submission FAN also referenced the recent survey by Li (2003). This is one of the studies that EPA (Baetcke et al, Nov 18, 2003) reviewed in a little more length. They wrote:

L. Li, The biochemistry and physiology of metallic fluoride: Action, mechanism, and implications; Crit. Rev. Oral Biol. Med 14: 100-114, 2003

EPA RESPONSE:

> "This publication is a review of information provided in the literature on the biochemistry and potential mechanism of action of fluoride complexes, particularly aluminum fluoride. The major focus is on the interaction of aluminum fluoride with Gprotein and the enzymes associated with G-protein activation. The review does not provide new information on the levels of fluoride or fluoride complexes that might be associated with adverse effects in laboratory animals or humans and thus, although potentially useful for hazard assessment, would not provide information useful for evaluation of dose-levels of fluoride associated with adverse effects."

None of these responses from the EPA suggests that they take this issue very seriously, largely because their primary focus appears to be to protect their MCL. Were they to take a larger view of their role in protecting the public they might pursue this matter with more vigor. What we have here is a general mechanism which may explain a number of toxic effects of fluoride on the body, all of which have been previously ignored, or downplayed, by the EPA with the exception of dental fluorosis and crippling

skeletal fluorosis. Even if they were to restrict their analysis to fluoride's impact on the bone they might well find that understanding fluoride's impact on G-proteins could explain fluoride's well known "anabolic" effect, which has potentially serious consequences.

Sodium fluoride has been used in the treatment of patients with osteoporosis in an effort to increase their bone mineral density by increasing bone turnover, but the treatment frequently leads to increased hip and other fractures. According to Caverzasio et al (1998):

> "Histomorphometric analysis iof iliac crest biopsies in osteoporotic patients receiving sodium fluoride for two years indicated that the change in cancellous bone volume induced by fluoride resulted from an increased number of osteoblasts and increased bone matrix synthesis."

Later in their paper Caverzasio et al suggest, based upon in vivo studies with rats, that

> "a fluoroalumino complex is probably the active fluoride molecule responsible for the enhancement of the proliferation of bone-forming cells and the change in bone mineral mass in vivo."

Caverzasio et al. hypothesize that aluminum fluoride [AlF4]- activates the G-protein (Gi), which triggers a cascade mechanism involving phosphorylation of the tyrosine side chains of several cellular proteins including extracellular signal-regulating kinase (ERK). ERK, once excited, results in cellular proliferation. As the name implies, ERK is normally excited by extracellular growth factors. Fluoride short circuits these normal growth factors, and thus may trigger cell division

To complain that the doses used in such experiments is high is not entirely accurate. Farley (1983) in laboratory studies found that fluoride can cause osteoblast proliferation at a serum fluoride level (2 micromole/Liter = 38 ppb) which is widely surpassed in fluoridated communities.

8. CENTRAL NERVOUS SYSTEM.

In FAN's submission we expressed many concerns with respect to fluoride's possible impact on the central nervous system and we subsequently submitted several other papers on this topic including Xiang et al. (2003 a and b).

a) Since the middle 1990s there have been several important studies which have probed fluoride's possible impact on the brain. Mullenix (1995) demonstrated that rats treated prenatally with fluoride showed behavior patterns associated with hyperactivity and rats dosed after birth showed hypoactivity. Guan et al. (1998) showed that membrane lipids in rat brain were impacted by chronic fluorosis.

b) Several studies in China (Lee et al. 1985 (sic - Li et al, 1995); Zhao et al. 1996; and Lu et al. 2000) have shown the possible impact of high background fluoride (possibly in the presence of low iodide, Zhao, 1998) on children's IQ. One of those that we have examined is the work by Zhao et al (1996) who found an approximate 5-10 point IQ deficit in children from a community with water containing 4 ppm natural fluoride compared to one containing 1 ppm. Since we fluoridate at 1 ppm, and the EPA's MCLG for fluoride is 4 ppm, this paper is of considerable concern.

c) An indication of how oblivious American authorities are to these possible dangers, is the fact that some schools in the US add 4.5 ppm of fluoride to their drinking water systems. For example, seventy-five (75) schools in Indiana fluoridate their drinking water systems at 4.5 ppm; sixty-one (61) schools (or communities) in North Carolina fluoridate their drinking water systems at 4.5 ppm; and sixty-four (64) schools in Kentucky fluoridate their drinking water systems at 4.0 ppm (US DHHS,1993). See TABLE 1.

d) Varner et al (1998) exposed rats to fluoride in their drinking water for one year. What was remarkable about this work is how low the concentrations were that caused damage. Both AlF3 (aluminum fluoride) and NaF (sodium fluoride) given to the animals at the level of 1 ppm fluoride (the same level generally used in public drinking water) in their doubly distilled de-ionized drinking water caused both kidney and brain damage, an accumulation of aluminum into the brain and the formation of amyloid plagues which are associated with Alzheimer's disease. Apparently, this is the third time that Isaacson and his co-workers have found effects on the brain at these remarkably low levels.

e) As a result of Varner's work aluminum fluoride was recently nominated by the Environmental Protection Agency and National Institute of Environmental Health Sciences for testing by the National Toxicology Program. According to the EPA and NIEHS, aluminum fluoride is a "drinking water contaminant" with "known neurotoxicity" and a "high health research priority." If fluoride is added to water which contains aluminum, than aluminum fluoride complexes will form (BNA, 2000, see http://www.fluoridealert.org/alum-fluoride.htm). We would add that if some of the fruits and vegetables with the fluoride residues proposed in DOW's application were cooked in aluminum saucepans, this too could lead to the formation of aluminum fluoride complexes.

The EPA's January 16, 2004 commentary focussed almost entirely on the issue of fluoride and Alzheimer's disease.

EPA RESPONSE:

> “The epidemiological and animal literature is insufficient to support a convincing association of fluoride exposure and Alzheimer's disease and impaired mental functioning. The Agency is not aware of any studies that provide a direct link between exposure to fluoride and Alzheimer's disease. In addition, one of the authors of the Varner (1998) study has indicated that the

> results of the study do not support a conclusion that aluminum or fluoride selectively damage the brain or that these compounds cause Alzheimer's Disease. The suggestion of an association made by the commenter is speculative and conjectural at this time (also see McDonagh 2000)."

The EPA commented on a number of the other studies we cited, either in the form of full critiques or in the tabled responses to all our references. These are all printed out below.

Mullinex, PJ, Denbesten, PK, Schunior, A and WJ Kernan. 1995. Neurotoxicity of sodium fluoride in rats. Neurotox. and Teratology 17 (2):169-177.

EPA RESPONSE:

> "Changes in behavior were monitored in groups of Sprague Dawley rats after exposure to fluoride at three different developmental stages. Body weight, plasma, and brain fluoride levels were also measured. Behavioral patterns such as sitting, grooming, head turns, standing, etc. were recorded by video camera and analyzed for their frequency and duration. Pairs of control and experimental animals were observed at the same time. Several of the statistical methods used to evaluate the data, e.g. the RS statistic and K(t) values, were not fully described by the authors...
>
> No significant differences were seen in the adults except for in the hippocampus in females and the medulla oblongata in males where the level in the treated animals were elevated. In the juvenile rats, the fluoride concentrations were increased significantly in most regions of the brain for both males and females. The authors concluded that the results of this study demonstrate the potential for fluoride exposures to influence the developing brain.
>
> The conclusions reached by Mullenix et al. (1995) are not supported due to a number of problems with their study. There have been no systematic studies comparing the Mullenix method for measuring neurobehavioral effects with the standard neurotoxicology battery, which has undergone extensive and international validation studies. There is no published record of validation of the Mullenix method. Also, the numerous T-Tests performed by these authors can lead to significance of results based on chance alone. Finally, there is no scientific basis to imply that motor changes are surrogate of cognitive deficits, as the authors do so in this paper."

EPA's criticism here hits a raw nerve of those who have followed this issue closely. Mullenix's paper was subjected by regulatory agencies in Washington, DC probably more intensively than any other comparable study in recent history. To say that her methodology was not subjected to "systematic studies" is sheer nonsense. Here is Mullenix's response (received by email March 21, 2004) to the three points made in the last paragraph of the EPA's response to her work:

There is no scientific basis for the following EPA comment: "There have been no systematic studies comparing the Mullenix method for measuring neurobehavioral effects with the standard neurotoxicology battery, which has undergone extensive and international validation studies. There is no published record of validation of the Mullenix method."

EPA blatantly distorts and ignores published, peer reviewed neurobehavioral literature. Even more surprising, is that it ignores literature that it paid for and helped to develop.

EPA needs reminding as to the origin of "the Mullenix method". The computer pattern recognition methodology used in my '95 NaF paper has a long pedigree. It started with the work done by Kernan & Higby (Energy and Mineral Resources Research Institute, Iowa State University in Ames Iowa), Hopper, Cunningham & Loyd (Veterinary Diagnostic Laboratory, Iowa State University) and L. Reiter (Neurotoxicology Division, Health Effects Research Laboratory, EPA-EPA Contract 68-02-2288) see Kernan et al., 1980 (Pattern recognition of behavioral events in the nonhuman primate. Computer Technology, C2-12) and Kernan et al., 1981 (Computer study of the behavioral effects of pharmacologic and toxicologic agents. Pharmaceutical Technology, 61-68, June).

I was a consultant on these original, EPA funded projects, and my contribution related to my extensive experience with behavioral observational techniques using time-lapse photograpy, a technique developed by Stata Norton at KU (personal friend of John Duell who was also on my thesis committee when I got my Ph.D.). I was the "human observer" of behavior that the physicists used to "train" a computer to recognize behavioral acts. The 1981 EPA sponsored paper described that the advantages of the new computer system were:

1)faster classification of behavioral observations
2)subjective human errors eliminated from behavioral observations and classifications
3)permanent record of data for faster analysis
4)capacity for measures not possible with conventional methods
5)improved accuracy
6)reduced interlaboratory variation in data

In the same paper they compared operant (used by Gary Whitford recently) and direct-observation methodologies (a computer pattern recognition system like I used). The following quote summarizes their comparison: "Since EPA is concerned ultimately with how chronic, low-level exposure to toxic substances affects behavior, the relative sensitivity of the direct observation and operant methodologies is extremely important. A significant question is: which of the two methods can detect behavioral changes at lower levels of exposure to a given substance? Research beyond the scope of the original EPA contract would be

needed to determine minimum concentrations that can be detected with the two methods. Direct observation, however, is as sensitive as operant procedures and in some cases is significantly ***more*** sensitive. At an exposure of 0.11 mg/kg of d-amphetamine, PROBE [pattern recognition of behavioral events] indicated dramatic changes in the location of the primate within the cage during the observation period, whereas delayed-response performance [operant method] for the same dose changed relatively little."

In short, EPA admitted over 20 yrs ago that direct observational methods were more sensitive than operant methodologies. Moreover, validation of direct observational methods is found throughout the medical literature, including validation of "the Mullenix method."

The original computer pattern recognition system (PROBE) was replaced with a new system (RAPID-Rodent activity pattern identification device-the one I used) because public sentiment turned away from the use of monkeys for large neurotoxic studies. The rat system was designed by Kernan again and me at Forsyth. **First**, we published how the computer identifies behavioral acts (Kernan, Mullenix & Hopper. Pattern recognition of rat behavior. Pharmacol. Biochem. Behav. 27:559-564, 1987). **Second**, we published how the computer quantifies behavioral data (Kernan, Mullenix, Kent, Hopper, and Cressie. Analysis of the time distribution and time sequence of behavioral acts. Internat. J. Neurosci. 43:35-51, 1988). **Third**, we expanded data analysis techniques (Mullenix & Kernan. Extension of the analysis of the time structure of behavioral acts. Internat. J. Neurosci. 44:251-262, 1989). **Fourth**, we demonstrated how the computer system generates dose-response data (Mullenix, Kernan, Tassinari, and Schunior. J. Am. Coll. Toxicol. 8:185-197, 1989.) **Fifth**, we demonstrated that the computer system could analyze the time structure of behavior and produce a more stable and reproducible measure that boosts sensitivity beyond that of other behavioral methods (Kernan, Mullenix & Hopper. Time structure anaysis of behavioral acts using a computer pattern recognition system. Pharmacol. Biochem. Behav. 34:863-869, 1989; Mullenix, Evolution of motor activity tests into a screening reality. Toxicol. Industrial Health 5:203-219, 1989; and Kernan & Mullenix, Stability and reproducibility of the analysis of time structure in spontaneous motor activity of male rats. Pharmacol. Biochem. Behav. 39:747-754, 1991).

The work by Kernan and myself at Forsyth with the computer pattern recognition system was taken up and investigated by the Iowa State Veterinary Labs to check or "confirm" every step/procedure developed at Forsyth. The Iowa Lab built their own RAPID system and confirmed our findings of its reliability, reproducibility, and reduction of data bias and error (Hopper, Kernan & Wright, Computer pattern recognition: An automated method for evaluating motor activity and testing for neurotoxicity. Neurotoxicol. Teratology 12:419-428, 1990.) The Iowa Lab went further and demonstrated that the RAPID system was more sensitive than other behavioral methods under nocturnal conditions (Hopper,

Kernan & Bowes, Reproducibility of time structure in motor activity of rats under nocturnal conditions. Pharmacol. Biochem. Behav. 42:245-250, 1992). In addition, Iowa tested it to see if it would detect the hypoactivity induced by the well known neurotoxin triethyltin (Kernan, Hopper & Bowes, Computer pattern recognition: Spontaneous motor activity studies of rats following acute exposure to triethyltin. J. Am. Coll. Toxicol. 10:705-718, 1991).

The following EPA comment also has no merit: "...the numerous T-tests performed by these authors can lead to significance of results based on chance alone." From the outset of developing the RAPID system, statisticians were consulted both at Forsyth and at Iowa State. In fact, the RS statistic (which we used in the '95 fluoride paper) was developed and validated by members of the Department of Statistics at Iowa State University (Kernan & Meeker, A statistical test to assess changes in spontaneous behavior of rats observed with a computer pattern recognition system. J. Biopharmaceutical Statistics 2:115-135, 1992.) As acknowledged in that paper, "The Monte Carlo simulations used in this study were very extensive. Approximately 9,000 analyses of each of the regular acts and the combined acts had to be done, and for each of these analyses every time distribution and time sequence analyzed had to undergo the 1000 repeated simulations for the bootstrap in order to estimate the standard deviation at each time point to assess whether that distribution or sequence was "changed." This required about 1400 h of CPU time on various DECstations, either 3100s or 5000s. This extensive effort could not have been done without the facilities of the Iowa State University Project Vincent distributed computer network." The data for this massive study was provided by me (at Forsyth) and Dr. Hopper (at Iowa), and the data from the two different laboratories were subjected to an in depth probe and comparative evaluation by the statisticians. EPA's simplistic dismissal of this impressive undertaking is a blatant disregard for scientific advances.

EPA made another unjustifiable comment concerning the Mullenix et al. '95 fluoride study: "Finally, there is no scientific basis to imply that motor changes are surrogate of cognitive deficits..." This comment is similar to one made by Proctor and Gamble scientists (Neurotoxicology and Teratology 17:685-686). My reply to that criticism still stands (Neurotoxicology and Teratology 17: 687-688, 1995). The scientific link between motor changes and cognitive deficits has been recognized for decades by many scientists and clinicians (Mullenix, The computer pattern recognition system for study of spontaneous behavior of rats. A diagnostic tool for damage in the central nervous system. In Motor Activity and Movement Disorders, Humana Press, 1995). We demonstrated that the RAPID system could detect changes in behavior that conventional operant methodology missed, i.e., the changes induced by agents that clinically are well known to cause cognitive impairment such as a lower IQ and impaired memory and attention (Mullenix et al. An animal model to study toxicity of CNS therapy for childhood acute lymphoblastic leukemia. Effects on behavior. Cancer Res. 50:6461-6465, 1990 and Mullenix et al. Interactions of steroid, methotrexate and

radiation determine neurotoxicity in an animal model to study therapy for childhood leukemia. Pediatr. Res. 35:171-178, 1994). Furthermore, based on findings using the Rapid System, we detected the role of steroids in neurotoxicity and predicted their relative impact on cognitive function. Our prediction was confirmed in clinical studies of children given steroid therapy for leukemia (Waber et al. Cognitive sequelae in children treated for acute lymphoblastic leukemia with dexamethasone or prednisone. J. Pediatr. Hemat. Oncol. 22:206-213, 2000). Operant methodology was not sensitive enough for studies of cognitive impairment associated with treatments for childhood leukemia.

Given the fact that other more recent studies support the findings of the Mullenix et al. '95 fluoride study, one would expect that EPA would be more scientific in its evaluation of our findings. It is clear, however, that something other than science is forcing EPA's head into the sand.

Dr. Phyllis Mullenix,
March 21, 2004.

(Ref.92) Guan ZZ, Y.N. Wang, K.Q. Xiao, D.Y. Dai, Y.H. Chen, J.L. Liu, P. Sindelar and G. Dallner. (1998). Influence of chronic fluorosis on membrane lipids in rat brain. Neurotoxicology and Teratology 20 537-542.

EPA RESPONSE:

"No brain histopathology and changes in membrane lipids above current MCLs."

This is another cavalier response from EPA.

(Ref.8) Calderon J et al. (2000). Influence of fluoride exposures on reaction time and visuospatial organization in children. Epidemiology 11(4): S153.

EPA RESPONSE:

"Abstract."

(Ref. 48) Li S, Zhi JL, Gao RO (1995). Effect of fluoride exposure on intelligence. Fluoride 28(4): 189-192.

EPA RESPONSE:

"Number of children with IQs below 70 or between 70 to 90 was greater in the medium- and severe-fluorosis areas than in the no- and slight-fluorosis areas. Children or the environment not monitored for exposure to lead, methyl mercury, or other contaminants that can influence mental development. Coal burning can contribute mercury to the environment as well as fluoride. Authors did not correct

> for potential confounding variables e.g., poverty, parental education, or other characteristics that might have influenced results."

These are all valid criticisms, many of which FAN has made itself, however this work has to be taken in conjunction with a whole series of studies that have come out of China on this issue. With each subsequent publication the authors have taken into account more and more of the confounding variable suggested by Western observers. The latest study by Xiang et al, is discussed below.

(Ref. 50) Lin FF et al. (1991). The relationship of a low-iodine and high fluoride environment to subclinical cretinism in Zinjiang. Iodine Deficiency Disorder Newsletter 7.

EPA RESPONSE:

> "Confounding factors-low iodine could have resulted in cretinism. No data on diet."

This is a very strange comment from EPA. The whole study was made in order to see if fluoride compounded the impacts of low iodide! Even when iodide was only moderately low the presence fluoride concentrations which were less than that added to the drinking water in the US (0.9 ppm versus 1 ppm) and certainly far less than the MCL, lowered still further IQs in children. This study is therefore of particular importance to children in the US of mothers who receive an inadequate amount of iodide in their diet. It is hard to fathom how EPA commentators can remain so blase about a finding which could be highly significant for millions of children.

(Ref. 51). Lu Y et al. (2000). Effect of high-fluoride water on intelligence of children. Fluoride, 33, 74-78.

EPA RESPONSE:

> "Other environmental exposures that can influence mental development were not accounted for in this study (e.g., exposure to lead, methyl mercury, other pollutants). The authors also did not account for confounding factors/variables, e.g., poverty, parental education."

See our comments for Li, Zhi and Gao above.

(Ref. 57) Morgan L et al. (1998). Investigation of the possible associations between fluorosis, fluoride exposure, and childhood behavior problems. Pediatric Dentistry - 20:4, 244- 252.

EPA RESPONSE:

> “Although refutes reference #58-by reporting no association between fluorosis, fluoride exposure and childhood behavior problems, this study alone can not resolve the issue of potential neurobehavioral problems. This study is not adequate to evaluate dental fluorosis and fluoride levels because it does not contain exposure data.”

The EPA's language here is revealing. The study "refutes reference #58". This is the language of debate or defense. Once again it is clear that EPA is engaged in a defense of a policy rather than an investigation of a problem. Reference #58 is actually the Mullenix study which has a highly different methodology: it was a rat study not a human study. FAN suspects that EPA meant reference #48, the Li et al study discussed above. More importantly, the criticism of this study is incorrect in our view. The severity of dental fluorosis is precisely the biomarker we should be using as a surrogate for fluoride exposure to children. It is far superior to fluoride levels in drinking water because children today are exposed to so many other sources of fluoride in addition to the fluoride in water. It is well established that the more fluoride a child is exposed to the more severe the resulting dental fluorosis will be. If the EPA and other agencies are determined to increase the levels of dental fluorosis in our kids, let's at least use it as marker for some decent scientific research.

Q Xiang et al., Effect of fluoride in drinking water on children's intelligence; Fluoride 36:84-94, 2003.

EPA RESPONSE:

> “A study by Xiang et al. (2003) measured the association between fluoride in drinking water and children's intelligence as measured by intelligence quotient (IQ). The study was conducted in two villages 64 km (40 miles) apart where the primary source of fluoride was from drinking water. Fluoride was measured in drinking water and urine with a fluoride ion selective electrode. Over 90% of the children, aged 8 to 13 years, in both villages (512 total, 222 in Wamiao and 290 in Xinhuai) participated in the study and received the Combined Raven's Test for Rural China which measures IQ. Both children and their parents supplied information on medical history (e.g., head trauma in the child), education level, socioeconomic status, and lifestyle. Urinary fluoride levels in children the Wamiao village, based on 155 samples, was 3.4 times higher than mean levels for children in the Xinhuai village (based on 135 samples). This difference correlated highly with levels of fluoride in drinking water. Mean IQ was 8 points lower in Wamiao than in Xinhuai, a statistically significant difference that was found in both males and females. "When compared with the children in Xinhuai . . . , the children in Wamiao exhibited, as the level of fluoride in the drinking water increased, a decrease in IQ and an increase in rates of mental retardation (IQ < 70) and borderline intelligence (IQ 70-79)". The correlation between IQ and level of fluoride in urine was statistically significant when measured directly and when adjusted for creatinine. Children in the village with low levels of fluoride showed

> an unexpected but significant decline of IQ with age. Family income and education level of parents did not correlate with children's IQ.
>
> The study by Xiang et al. (2003) is severely deficient in its discussion of potential biases and confounders. The discussion section mentions the lack of association with family income and education, but does not discuss any other potential confounders. Lifestyle is mentioned in the methods but no results or discussion is presented. No explanation is provided for the unusual decline of IQ with age in the low fluoride exposed village, besides fluoride. No consideration was paid to where children lived within the village and how other factors might affect IQ. Known confounders such as intermarriage between close relatives are not discussed. The well known effect of parent's IQ was not measured except by education level which did not correlate suggesting this measure was inadequate for that purpose. Given the lack of exploration of other possible causes for the pattern of IQ, little weight can be placed on the results from this study. The study does mention 3 other studies which apparently support the current findings. These other studies are inadequately discussed in a single sentence without any mention of their strengths and weaknesses. Though the authors have apparently made a careful statistical analysis, their epidemiologic analysis is too incomplete to warrant any conclusion until other contributing factors, confounders, and biases are fully explored."

Once again we see EPA absolutely determined to nitpick every study which remotely impeaches fluoride of causing harm. This approach to research on a controversial issue is very reminiscent of the barrage of attacks that greeted every new study that implicated sub-clinical lead levels as causing a lowering of IQ and behavioral problems in children which appeared in the 1970's. We now know that the barrage of attacks on this issue were orchestrated by the lead and gasoline industries which did not wish to see a phase out of lead tetraethyl. Eventually of course, Needleman et al (1979) clinched the issue.

This study by Xiang et al has to be seen in the context of at at least three other studies from China which have found the same findings: namely a lowering of IQ in children with higher exposure to fluoride. Because EPA seems to rely on FAN for nearly all its references on this issue, it was unaware that subsequent to this paper by Xiang and co-workers, they sent another short article to Fluoride, which showed that they had controlled for blood lead levels. Thus these workers have controlled for at least two very important confounding variables which could influence this result - iodide and lead. Admittedly they didn't look into methyl mercury levels or the levels of intermarriage. Hopefully EPA will communicate these concerns to the Chinese Public Health Service, meanwhile a weight of evidence analysis of the biochemical, animal and human studies available is highly suggestive that fluoride could damage the developing brain of young children. If we couple this information with the knowledge that evolution put very litte fluoride into mothers' milk (0.005 -0.01 ppm) one has to wonder about a government which continues to uphold a drinking water standard (MCL = 4 ppm) based upon the

end point of crippling fluorosis while allowing fluoride to go into the bodies of infants and young children at 400 to 800 times the level nature intended.

(Ref. 80) Zhao LB et al. (1996). Effect of high-fluoride water supply on children's intelligence. Fluoride, 29, 190-192.

EPA RESPONSE:

> "There were more children with IQs less than 80 in the high fluoride village (25) than in the low fluoride village (9). As was the case with the study by Li et al.(1995), the potential for exposures to chemicals e.g., lead and methyl mercury which have a demonstrated effect on IQ was not assessed and the data were not corrected for possible confounding variables."

This is one of series of papers from China on the same issue and our comments on li et al., lu et al. and Xiang et al. apply here.

(Ref. 75) Varner JA et al. (1998). Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water; alterations in neuronal and cerebrovascular integrity. Brain Research, 784, 284-298.

EPA RESPONSE:

> "Before the results of this study are used for drawing inferences regarding human risk, this study needs to be repeated due to several major limitations, e.g., absence of dose-response assessment and evaluation of functional impairments, and lack of assessment of fluoride in the animal chow. With respect to the renal effects, it is important to distinguish those caused by aging versus fluoride treatment."

Again, EPA treats another very important study very lightly. Varner and coworker's findings are remarkable. They found feeding rats fluoride in their drinking water fluoride at 1 ppm (administered either as aluminum fluoride or sodium fluoride) for one year caused:

a) kidney damage
b) brain damage
c) accumulation of fluoride in the brain
d) beta amyloid deposits which are characteristic of Alzheimer's disease.

When EPA says this study needs repeating they are correct, but they don't acknowledge that the authors themselves have repeated the finding three times. Nor did they indicate if they, or any other agency, is currently pursuing or funding such studies. Varner's work was funded from private sources. What they describe as major

limitations are not, they are more examples of EPA nitpicking. We will discuss them one at a time:

1) An absence of dose-response assessment. The authors found a response at the lowest dose they used and less of a response at higher doses. This is unusual but it is not unique. If correct it may cause reevaluation of other animal studies, like those of Sprando and Collins (discussed in section 9) who may have used too high a dose over too short a period.

2) When they talk about an "absence of functional impairments" are they suggesting that beta amyloid deposits are of no or little "functional" consequence? If they are they are being plain silly.

3) Lack of analysis of fluoride in the animal chow is something that needs to be corrected in future studies, but unless they have reasons to believe that the control rats did not have the same chow the argument has no merit.

4) When they argue that "With respect to the renal effects, it is important to distinguish those caused by aging versus fluoride treatment" isn't that what you normally expect to tease out using control animals, or are they suggesting that the controls aged at a different rate from the test animals?

Clearly, this study which has found dramatic effects in rats fed 1 ppm fluoride in their water is a very threatening one for those who wish to maintain that 4 ppm of fluoride in the drinking water is perfectly safe for humans to drink for a whole lifetime.

(Ref.81) Zhao, W., Zhu, Z, Yu, Z, Aoki, K, Misumi, J, and X Zhang. 1998. Longterm effects of various iodine and fluorine doses on thyroid and fluorosis in mice. Endocrine Reg 32:63-70.

EPA RESPONSE:

> "The authors of this report studied the interactions of fluoride and iodine in Kunmin mice. The animals were divided into nine groups of 32 animals which received different combinations of iodine and fluoride in their drinking water. Iodine was administered as potassium iodate and classified as iodine deficient (ID , 0 µg/L), iodine normal (IN, 20 µg/L) or iodine excess (IE, 2500 µg/L). The drinking water fluoride concentrations (NaF) were: fluoride deficient (FD, 0 mg/L), fluoride normal (FN, 0.6, mg/L) or fluoride excess (FE, 30 mg/L). A special low iodine and low fluoride chow was fed the animals. The nine combinations of nutrients were ID/FD, ID/FN, ID/FE, IN/FD, IN/FN, IN/FE, I.E./FD, I.E./FN, and I.E./FE. The animals were treated for 150 days and the following parameters were measured, incisor fluorosis at two-weeks, radioiodine uptake at 100 and 150 days, Serum T3 and T4, histological evaluation of the thyroid, fluoride content of the bones.

For many of the nutrient combinations there were no apparent interactions between fluoride and iodine. Fluoride produced the expected incisor fluorosis at two weeks in the FE group. There was no incisor fluorosis in the FD or FN groups. An ID increased the severity of fluorosis in the FE group. The fluoride content of bones was dramatically increased in the FE group. Surprisingly it was higher in the FD/ID group than in the FN/ID group. Otherwise the FD and FN groups with IN or IE exposures has fairly similar bone F levels.

Under ID conditions increasing F intake appeared to be associated with a decrease in thyroid weight. At 100 days the incidence of goiter in the ID group increased with increasing F intake. When the iodine was in excess, the incidence of goiter decreased with increasing F intake. Increased T3 levels were seen in the ID/FE groups and T4 was decreased in the ID/FD and ID/FN groups. FE inhibited radiolabeled iodine uptake in the ID and IN groups.

The mechanistic basis for the interactions between fluoride and iodine observed in this study is not clear. The authors mention that their T3/T4 results are not consistent with other studies and acknowledge that they can not offer any plausible explanation between their results and those of other investigators. The authors also state "it is generally believed that fluorine does not influence either thyroid function or structure at the amount (about 1ppm in water) used to prevent dental caries." The authors suggest the possibility of interactions between the two minerals, particularly when deficiency of one is combined with excess of the other. The mechanistic basis for the interactions between fluoride in iodine observed in this study is not clear."

9. REPRODUCTIVE EFFECTS.

A. HUMAN STUDIES.

Freni SC. (1994). Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. Journal of Toxicology and Environmental Health, 42:109-12.

In FAN's submission we pointed out that Freni, a researcher at the National Center of Toxicological Research (NCTR), the research division of the FDA, found a statistically significant reduction in fertility in counties with 3 or more ppm fluoride in the water. Clearly, this is a very important study in terms of the adequacy of the MCL of 4 ppm. We pointed out that there had been some criticism of Freni's methodology but that none of it had been published. In this respect, it is curious to note that the ADA in their booklet "Fluoridation Facts" cites the criticism based on " a personal communication" from Dr. John Sinks, dated Nov 6, 1992, which is two years before Freni's study was published!

EPA's specific response to Freni's work appears in Table 1a of Baetke et al (2003) p 50. They write:

> "Study did not measure factors affecting fertility, e.g., contraceptive practices. Cohort effect prior to 1980 not measured by use of single point estimates for key measures of sociodemographic status. An ecologic study that measured county levels of fluoride rather than individual level and cannot be used to show a causal association. Author acknowledges need for follow up study on individual women to verify results.."

However, when we contacted Dr. Freni to seek his reaction to this and the previously unpublished criticism of his work, he wrote to us that,

> "In the US, I was employed by the DHHS, from 81-88 at the Centers for Disease Control (CDC), and from 88-96 at the National Center for Toxicological Research (NCTR), which is the research center of the FDA. At both agencies I served as the specialist in Risk Assessment of environmental exposure, and as such I have served in several committees, among others a subcommittee appointed by the Committee to Coordinate Environmental Health and related Programs (CCEHRP) to review the literature on the benefits and risks of fluoride exposure. I represented NCTR in that body and, as far as I can remember I was the only MD, and surely the only epidemiologist. Given my background I focused on the Risks, leaving the Benefits to the others. The result of my literature study was unexpected: all animal species studied revealed reproductive damage. There was no human study on reproductive effects. The subcommittee's report had to be unanimous. I was asked to tone down my review of animal studies so as to not alarm the public. My review itself was not criticized. After all, it was based simply on a review of peer-reviewed published papers. I refused to water down my report, and the committee decided to do it for me. The result is pp. 66-67 of the "Review of Fluoride: Benefits and Risks, Report of the Ad Hoc Subcommittee on Fluoride, of the Committee to Coordinate Environmental Health and Related Programs CCEHRP), Public Health Service, Department of Health and Human Services (DHHS), February 1991).
>
> Eventually, I decided to independently start a study of the reproductive effects of Fluoride in humans. Given the lack of funding for a multidecade long study (extremely expensive), I designed a so-called "ecological study", knowing full well its shortcoming, as acknowledged in the paper. In this, I was fully supported by my agency. By the way, the F-levels in water were provided by the CDC despite their resistance to my planned study. Against my expectations, the study fits what was found in animals.
>
> The paper was peer reviewed and unanimously approved. I have received no communications on the paper's content or conclusions from any critic, including Dr. Sinks. What I did receive were numerous requests for a reprint from all over the world, nearly all from public health institutions and universities. The study is

> certainly not flawed, NCTR checked the statistics thoroughly. What can be criticized is the fact that it is an ecological study. So what: the alternative is to close your eyes and not to do any study at all, or to finance a neutral body (is there any?) to conduct a full follow-up study. I was starting on an expansion of the study to include low-birth weights, premature births, and stillbirths as outcomes. Unfortunately, I fell ill and eventually decided to return to Europe." (Dr. Stan Freni, Personal communication, email March 19, and editorial changes by phone March 21, 2004).

We would add that if EPA insists on knocking out all ecological studies they would eliminate the vast majority of the studies which purport to demonstrate that fluoride reduces tooth decay! This is yet another double standard. Moreover, such arguments have a particularly hollow ring from government agencies which have made absolutely no effort to collect data in the form of bone and serum levels in a comprehensive fashion. This would be the most appropriate way of establishing individual exposure and provide the basis for more meaningful epidemiological work on many end points.

In appendix F, FAN has reprinted the review of animal studies on reproduction by Freni which he published as the introduction to his 1994 paper. This was based on the review he provided for the subcommittee on Fluoride of CCEHRP. We contrast this with the review which was substituted for Freni's review in the final report published by the DHHS in 1991. According to Freni this was toned down so as "not to alarm the public."

Other human studies on reproductive effects which have appeared since the 1993 NRC review, submitted by FAN and criticized by the EPA (Baetcke et al. Nov 18, 2003) were:

Susheela AK, Jethanandani P (1996). Circulating testosterone levels in skeletal fluorosis patients. J Toxicol Clin Toxicol, 34(2): 183-9.

EPA RESPONSE:

> An epidemiological study comparing testosterone levels in 3 groups: fluorosis patients with 3.9 ppm F in their water, controls with high F in their water(4.5 ppm) and controls with low F in their water (0.5 ppm). According to the authors, fluorosis patients had the lowest serum testosterone, with the high water F controls. However, confounding factors (e.g., age, diet, health status, exposure to other chemicals) were not accounted for that could affect testosterone levels.

Michael M, Barot VV, Chinoy NJ (1996) Investigations of soft tissue functions in fluorotic individuals of North Gujarat Michael M, Barot VV, Chinoy NJ Fluoride 29:63-71.

EPA RESPONSE:

> "This is a survey of clinical chemistries among residents in a region of India with

> high levels of fluoride in drinking water (500 people from 52 villages; 1.0 - 6.5 ppm fluoride) compared to persons in a 'normal' fluoride level city (mean 0.6 ppm). The number of individuals used to compute summary data in the paper varied from 40 - 76 in the high fluoride group and 15 or 22 in the normal fluoride group. Dental and Skeletal Fluorosis were common in the high fluoride residents: 74% had slight to severe tooth mottling; 59% had stiff spine. There was a correlation between water fluoride and serum fluoride concentrations. Serum fluoride also showed a significant age relationship.
>
> Differences were found for some clinical chemistries in the high fluoride group compared to the normal group. Significant decreases were noted for protein and calcium concentrations. Significant increases were noted for SGOT, SGPT, sodium, potassium, adrenalin, noradrenalin, cholesterol and T4. No changes were noted for T3, TSH, hemoglobin and testosterone."

This final comment by the EPA is not quite accurate. What the authors actually reported was:

> "Circulating levels of testosterone were decreased, but not significantly enough to indicate an effect on reproductive functions. The enhanced levels of serum transaminases, which are markers for liver function, indicated structural and functional changes in liver due to fluoride intake. Changes in the serum calcium, sodium and potassium levels revealed electrolyte imbalance in the fluorotic individuals. While levels of thyroid stimulating hormone (TSH) and triiodothyronine (T3) did not vary, a significant increase in the thyroxine (T4) levels suggested alteration in thyroid function. Thus
> the study revealed some harmful effects of fluoride in the soft tissue functions of the endemic population."

Once again we see an attempt by EPA to downplay an important finding. First, the levels in the community examined (1 - 6.5 ppm) were not "high", they actually embrace the current MCL of 4 ppm and second, at these levels, they found some "harmful effects of fluoride in the soft tissue functions."

D. Ortiz-Perez et al. (2003), Fluoride-induced disruption of reproductive hormones in men; Environ. Res. 93:20-30.

EPA RESPONSE:

> Ortiz-Perez et al. (2003) studied the association between fluoride exposure and subclinical effects related to reproductive hormones. This study looked at 133 men aged 20-50 years with occupational exposure to fluoride and compared to 27 men whowere only exposed to fluoride in drinking water...
>
> The doses reported in this study were higher than in the United States and Canada but lower in Russia and India where fluoride has been associated with

other effects on hormones (notably lowered testosterone) and reproductive effects (male infertility).

This study suffers from a poor design and inadequate sampling. Nothing is said about how the 27 workers exposed only to drinking water were selected, other than that all subjects apparently resided in the same town. The only selection criteria for exposed workers appears to be at least one year employment in plants where fluoride exposure occurred. In the occupational group, the authors fail to acknowledge that lower exposed administration workers undoubtedly differed in many lifestyle factors (e.g., diet, access to health care, medical history) from the other more highly exposed workers and therefore, are inadequate for use as an internal comparison. The authors acknowledge that other exposures at the plant may be an explanation for some of their findings. How long workers resided in the town, types of occupations among the reference population, lifestyle differences, environmental exposures, and other factors that might influence hormonal levels are inadequately or barely discussed.

The authors fail to acknowledge that all the major factors which influence hormonal levels in men have yet to be identified and their study may have easily missed important factors which influence reproductive hormones. It must be emphasized that all of the effects measured were subclinical and that this study did not find evidence of an adverse reproductive outcome. This study did suggest a possible influence of fluoride manufacturing exposures on a hormone level that may be consistent with effects reported in other studies. However, this study is much too preliminary and no conclusions about the effects of fluoride on hormone levels are warranted until this study is repeated in other populations with a carefully chosen comparison group and sufficient sample size to analyze for the effects of confounding variables.

While the EPA is correct in stating that not all confounding variables were taken into account (they seldom are in most human studies)in this study, the fact remains that the authors found a statistically significant relationship between urinary fluoride levels and lowered testosterone levels. Taken together with the findings of Susheela et al(above); Michael et al (above); previous reports from Russia and numerous animal studies among several species (discussed below) a weight of evidence analysis would suggest a highly plausible relationship between excessive fluoride exposure and the lowering of testosterone levels in men. This lowering might well explain Freni's findings in counties with water fluoride levels at or above 3 ppm.

ANIMAL STUDIES.

In Freni's 1994 study (discussed above) he makes it clear that the driving force behind examining a possible relationship between fertility and fluoridation in the US population was the huge amount of animal data which indicated fluoride's ability to impact the reproductive system in many different species. It is particularly intriguing to note the sharp contrast between Freni's survey on this matter and the survey presented by the

DHHS in its 1991 report Health Effects of Fluoride: Benefits and Risks, because Freni actually served on the panel that drew up this report:

EPA comes down on the same side as the DHHS reviewer and places far greater emphasis on the few studies which have been unable to demonstrate reproductive effects rather than on the very many which have, they write:

> "... there is insufficient evidence to establish a causal link between fluoride exposure and effects on reproductive function in humans. Adverse effects on reproduction are primarily reported from animal studies (e.g., Reference #14 Chinoy et al. 1988). Although the Chinoy paper reported adverse effects in the testes, these effects were not reproduced by other laboratories (articles not cited by FAN: For example see:Collins TF, Sprando RL, et al, Multigenerational evaluation of sodium fluoride in rats Food Chem Toxicol. 2001 Jun;39(6):601-13.; Sprando RL, Collins TF, et al., Black TN, Rorie J, Ames MJ, O'Donnell M, Testing the potential of sodium fluoride to affect spermatogenesis in the rat., Food Chem Toxicol. 1997 Sep;35(9):881-90; Li Y, Dunipace and Stookey, Effects of fluoride on the mouse sperm morphology test, J Dent Res. 1987 Sep;66(9):1509-11.).

FAN Response:

We disagree. There is sufficient published data available for EPA to respond to the adverse effects of fluoride on the male reproductive system. The references EPA cites are not sufficient for dismissing this concern.

Collins, Sprando et al.

EPA cites the June 2001 "Multigenerational evaluation of sodium fluoride in rats" by Sprando, Collins et al. (Food Chem Toxicol. Jun;39(6):601-13). For such an important study the authors presentation of data is unsatisfactory. This is one of 2 multigenerational studies published by the authors in 2001. For both studies:

- No Fluoride blood levels are presented
- No Fluoride levels in bone or organs are presented
- Skeletal and Sternebral variations are not identified by sex
- Incidence of specific soft -tissue variations are not identified by sex
- Runts are not identified by sex
- Spontaneous disease lesions and incidental findings are not defined.

Sprando, Collins et al. published 6 papers in ***Food and Chemical Toxicology*** (1995, 1996, 1997, 1998, and two in 2001) in their study of the effects of fluoride. Not one of these papers presented fluoride levels in blood or organs.

Without the fluoride levels in blood, bone, and organs, one cannot fully understand the results presented, or dismiss as 'random' the high number of effects in the Control group compared to all treated groups in the their multigenerational studies. Adding to the concern of the high number of effects found in the Control group is the fact that the rats utilized in the 1997 and 1998 published studies were obtained from the multigenerational studies. According to the authors:

> 1997 paper, p 882: "Male rats utilized in this study were obtained from a larger 2-generation reproduction study." Testing the potential of sodium fluoride to affect spermatogenesis in the rat.
> Sprando RL, Collins TF, Black TN, Rorie J, Ames MJ, O'Donnell M. Food Chem Toxicol. 1997 Sep;35(9):881-90
>
> 1998 paper, p 1118: "The 25 male rats utilized in this study were obtained from a larger two-generation reproduction study." Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study. Sprando RL, Collins TF, Black T, Olejnik N, Rorie J. Food Chem Toxicol. 1998 Dec;36(12):1117-24)

In the second 2001 study (pp 867-876), with fluoride treated groups at 25-ppm, 100-ppm, 175-ppm, and 250-ppm, with no fluoride levels in bone or blood presented, the following results were found:

> • Incidence of specific skeletal variations (excluding sternebral) for F2 fetuses (Table 7).
> -- Of 25 Parameters listed: the Control group had higher effects than the 25-ppm and 100-ppm treated groups.
> -- Of 25 Parameters listed: the 250-ppm treated group had one higher effect than the Control group (11 vs 10 respectively)
>
> • Incidence of specific sternebral variations in F2 fetuses (Table 5):
> -- Parameter: Reduced ossification: the Control and 250-ppm treated group had the same number of effects.
> -- Parameter: Non-ossified: the Control had higher number of effects than the 250-ppm treated group

The rats used in the 1995 study were obtained as adults from the Charles River Laboratories. No mention is made of the diet they were fed prior to being obtained by Sprando, Collins et al. No fluoride levels in blood, bone or organs are

presented. The Controls in this study also had a high number of effects compared to treated groups. Some examples:

• Analysis of incidence of soft-tissue variations in foetuses (Table 9).
-- The Control group had the highest number of effects compared to all NaF treated groups (10-ppp, 25-ppm, 100-ppm, 175-ppm, and 250-ppm)

• Incidence of specific sternebral variations in foetuses (Table 4).
4 of the 6 Parameters listed in this Table:
-- Parameter: Mishapen: The Controls had the highest number
-- Parameter: Malaligned: The Control had higher effects than the 10-ppm, 25-ppm, 100-ppm, and 175-ppm fluoride treated groups
-- Parameter: Incomplete ossification: The Control had higher effects than the 10-ppm, 25-ppm, and 175-ppm fluoride treated groups
-- Parameter: Non-ossified: The Control had higher effects than the 10-ppm, 100-ppm, and 175-ppm fluoride treated groups

• The control group showed the highest occurrence of ***in utero*** deaths per litter (Table 3).

Ref: Developmental toxicity of sodium fluoride in rats. Collins TF, Sprando RL, Shackelford ME, Black TN, Ames MJ, Welsh JJ, Balmer MF, Olejnik N, Ruggles DI. Food Chem Toxicol. 1995 Nov;33(11):951-60.

Of the rats used in the 1996 study, all that is mentioned is: "96 adult male Sprague-Dawley rats weighing 225-460 g were used. No mention is made of where these rats were obtained or the diet they were fed prior to being obtained by Sprando, Collins et al. No fluoride levels in blood or organs are presented.
Ref: Effect of intratesticular injection of sodium fluoride on spermatogenesis. Sprando RL, Black TN, Ames MJ, Rorie JI, Collins TF. Food Chem Toxicol. 1996 Apr;34(4):377-84.

Lastly, Sprando, Collins et al. are consistent in stating in all but their 1996 paper that the rats were fed the low-fluoride NIH-07 diet (7.95 ppm luoride), "the diet for the fluoride studies conducted by the NTP (NTP 1990)."

In 1991, Robert Carton, representing US EPA's headquarters Professional's Union, Local 2050, wrote about the problem with Controls used in the National Toxicology Program (NTP) in 1990. Carton wrote:

.... The most important observation: the control animals were not fed fluoride-free diets. Also, the animals were four weeks old at the beginning of the study and obviously not fluoride free. Male rats were fed a diet containing 7.9 ppm or 0.2

> mg/kg/day of fluoride, about six times the dose received by a human drinking 2 liters/day of water containing 1 ppm fluoride. Animal studies have been successfully completed with a diet containing 0.05 ppm fluoride. The historical controls had even more fluoride in the diet. NTP estimates that normally their standard feed has from 28 to 47 ppm, or about 0.7 to 1.2 mg/g/day. The study had this to say about the dose concerning historical controls:
>
> Assuming a maximum bioavailability of 60%, the historical database animals actually constitute a group receiving sufficient fluoride to place them between the low- and mid-concentration groups in the current 2-year studies.
>
> This is an excellent argument for excluding the use of historical controls as a comparison group anywhere in the report. Yet, time and time again, they are used to compare with the results of the study and often it is to downgrade the results. ...
>
> Ref: Fluoride 1991; 14(3):85-89
> Editorial: **National Toxicology Program - Critique of Peer Review Draft Report**
> RJ Carton
> Representing the Professional's Union, Local 2050, National Federation of Federal Employees (at the Environmental Protection Agency headquarters), Washington DC 20046. USA.
> Excerpts available at: http://www.fluorideaction.org/pesticides/1991.f.abstracts.htm

In summary**,** until fluoride levels in blood, bone and organs are published from the Sprando, Collins et al. studies, a full understanding of their results is not possible. It should also be noted that the methods Sprando, Collins et al. used in their male reproduction studies differ from the majority of other studies that have found effects on the male reproductive system. This should not be used to invalidate any of the studies that have found effects (see Appendix G). Also, the 6 studies by Sprando, Collins et al. used only the rat.

EPA states

> "Although the Chinoy paper reported adverse effects in the testes, these effects were not reproduced by other laboratories"

We have explained our concerns with the Sprando, Collins, et al. studies above. However, in a 1998 paper (not cited by EPA), Sprando et al. report the following:

> ... A statistically significant decrease in the absolute volume and volume percent of the lymphatic endothelium was observed in the 175 and 250

> ppm NaF-treated groups and in the testicular capsule in the 100 ppm NaF-treated groups. The significance of this finding is unknown at the present time...
> The inability of NaF to produce reproductive effects in the present study could be attributed to the following: (1) species sensitivity to NaF expsoure; (2) the dose and route of exposure; or (3) the resistance of the strain of rats used in the present study to testicular toxicants... It is more likely that species sensitivity, dose and route of exposure are the primary reasons for obtaining equivocal results. Carefully controlled comparative studies should be designed to examine species sensitivity and routes of exposure...
> Ref: Testing the potential of sodium fluoride to affect spermatogenesis: a morphometric study.Sprando RL, Collins TF, Black T, Olejnik N, Rorie J. Food Chem Toxicol 1998 Dec;36(12):1117-24

Li, Dunipace and Stookey

As to the 1987 paper that EPA cites: "Effects of fluoride on the mouse sperm morphology test," by Li, Dunipace and Stookey published in the Journal of Dental Research. The authors evaluated the "genotoxic effects of fluoride" and reported: "The results of this study showed that NaF did not have adverse effects on mouse sperm morphology."

In the same year, 1987, Pati and Bhunya reported very different results in mouse sperm in their paper, "Genotoxic effect of an environmental pollutant, sodium flouride, in mammalian ***in vivo*** test system" (Caryologia, 40:1-2; 79-87). They state:

> Genotoxicity of Sodium fluoride was evaluated in mice in vivo with the help of different cytogenetic assays. The frequency of chromosome aberration was dose - and time - dependent but not exactly route-dependant. Fractionated dosing induced less aberration. Incidence of micronucleus and sperm abnormality increased with dose. Relative sensitivity of the three assays has been found to be: Sperm abnormality > Chromosome aberration > Micronucleus. The present results have revealed mutagenic property of NaF. ... Of all the assay results in the present study, the sperm abnormality was highest and incidence of MN was least. Such result is not unexpected since greater sucess rate of sperm abnormality over other assay results in detecting agents that are active ***in vivo*** has been reported for many chemicals (Bruce and Heddle 1979).

Medical Research Council Working Group Report (MRC 2002)

Additionally, EPA cites the comments of the Medical Research Council Working Group Report (MRC 2002), that the

> "plausibility of fluoride affecting the reproductive capacity of humans at the intakes experience from fluoridated drinking water is low."
> - page 32 of report, available at
> http://www.nofluoride.com/reports/MRC_Fluoridation_Report.pdf

It should be noted:

-- The potential for effects from life-long exposure to, and accumulation of, fluoride in humans is the concern. The majority of studies indicate that fluoride has a definite adverse effect on male reproduction. These studies should not be dismissed, especially in light of the little we know for the reasons for infertility. According to Larry I. Lipshultz, M.D., Professor of Urology, Baylor College of Medicine, Houston, Texas.:

> The testes, vulnerable to systemic and environmental insult, play a vital role in a man's ability to father a child. Understanding this role is an important part of the complex process of evaluating and treating the growing numbers of men seeking treatment for male infertility. The number of couples affected by infertility is currently estimated to be 15% of all couples attempting to have children. The difficulties are attributable to a significant male factor alone in 30% of couples and to a combination of male and female factors in an additional 20%. Therefore, in 50% of all infertile couples, an abnormal male factor contributes to reproductive failure.
> Ref: Male Infertility: Etiology, Investigation, and Treatment.
> http://www.bcm.tmc.edu/urol/fertility/pteval.htm

-- the MRC Report references three Sprando, Collins et al. reports to support this statement. With the concerns we expressed above, it must be noted that the MRC report did not reference any of the reports we present in Appendix G (on Male Reproduction Studies)

-- the MRC Report relies heavily on the York Report (2000) http://www.york.ac.uk/inst/crd/fluorid.pdf, which did not consider any of the papers in Appendix X

EPA's dismissal of the published papers FAN submitted on reproductive effects

EPA's dismissal of the published results we submitted on reproductive effects is unfortunate in light of the papers they cite to refute them.

However, we do agree with some of the comments EPA have made on the papers we submitted previously. We have withdrawn the following papers in response to comments from EPA:

> EPA: In the study by Chinoy, et al. (Microdose vasal injection of sodium fluoride in the rat, 1991) direct injection into the vas deferens was used which is not a relevant route of human exposure, and thus should not be used for dose-effect extrapolation.
> FAN: We agree. This study was conducted to verify other findings from this laboratory.

Of the many animal studies we present in Appendix G, methodologies and species vary. When studies report conflicting results it does not necessarily invalidate all results. EPA should consider that each method may be unique in its sensitivity to finding adverse effects. The fact that effects on the male reproductive system have been found in experiments with several animal species (rats, mice, rabbits, gerbils, guinea pigs, bank voles) has significance.

Papers submitted to EPA for review

Appendix G presents the published papers that report adverse effects on the male reproductive system.

We urge EPA to consider the preponderance of the published reports that indicate harm to the male reproductive system from fluoride exposure.

EPA appear to be unaware that Dr. Chinoy, the leading researcher in the field of fluoride's effect on the male reproductive system, is one of many independent scientists who have found definitive adverse effects on the male reproductive system - see Tables 1 and 3 below. In 1997, Chinoy et al. stated that twelve studies from their laboratory

> "revealed that fluoride, one of the naturally occurring toxicants, affects the structural and functional integrity of reproductive organs in male and female rodents and guinea pigs, and leads to the loss of fertility."

Ref: Fluoride toxicity on rat testis and cauda epididymal tissue components and its reversal NJ Chinoy, S Shukla, AS Walimbe, S Bhattacharya Fluoride, Vol 30, No. 1: 41-50. 1997.

Page 24 of 55 (Baetcke et al, Nov 18, EPA)

E.H. Goh and A.W. Neff, Effects of fluoride on Xenopus embryo development; Food and Chemical Toxicology 41:1501-1508, 2003

EPA RESPONSE:

The effects of sodium fluoride (NaF) on the development of frog embryos were evaluated in this study. The concentrations of NaF used ranged from 0 to 20 mM. The authors of this study concluded that NaF acted as a teratogen and reported that most prominent malformations caused were reduction in the head-tail lengths and dysfunction of the neuromuscular system of tadpoles. It should not be concluded from this study that NaF also acts as a teratogen in mammalian fetuses to cause malformations. It should be noted that in a recent developmental toxicity of NaF by the Food and Drug Administration (Developmental toxicity of sodium fluoride measured during multiple generations. Collins TF, et al., Food Chem Toxicol. 2001 Aug; 39(8):867-76.), no dose-related anomalies or malformations in internal organs were observed. Additionally, numbers of corpora lutea, implants, viable fetuses and fetal morphological development were similar in all treatment groups. The only effect found was decreased ossification of the hyoid bone at 250 ppm (above the current MCL). FDA also evaluated NaF for reproductive effects and found no cumulative effects in three generations. (Multigenerational evaluation of sodium fluoride in rats. Collins TF, et al., Food Chem Toxicol. 2001 Jun;39(6):601-13). Furthermore, this frog embryo assay can not be used for the quantitative extrapolation of human potential risks. The FETAX assay is currently being reviewed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), where it is being proposed only as a "screening method" for evaluating the developmental toxicity potential of chemicals.

It's unfortunate that the EPA reviewer doesn't even express concern for the teratogen findings in the Goh and Neff report, but instead attempts to dismiss them with citing Sprando, Collins et. al., which we have just discussed. In the Final Rule of January 23, 2004, EPA reports the following adverse effect from a **sulfuryl fluoride rabbit study**:

Prenatal developmental--**rabbit** (870.3700)
Developmental
NOAEL = 75 ppm or 29/29 (M/F) mg/kg/ day
LOAEL = 225 ppm or 86 (F) mg/kg/day
based on decreased fetal body weight, **decreased crown-rump length ...**

EPA's critique said that Goh and Neff's results on frogs shouldn't be extrapolated to mammals, even though the authors said that this was a "strong possibility." The rabbit study reported in the Final Rule describes the same effect as Goh and Neff, which they described as **"a reduction in the head-tail lengths**."

10. OSTEOSARCOMA AND CHROMOSOMAL DAMAGE.

In FAN's submission we drew attention to the mixed evidence on fluoride's ability to cause genetic damage and cancer, particularly osteosarcoma in young men. The EPA paraphrased our comments:

The likelihood of fluoride acting as a genetic cause of cancer must be considered. Although NTP found no increased cancers in the mice study, they found a dose related increase in bone cancer (osteosarcoma) in the male rats. They described this as "equivocal evidence of carcinogenicity." A national cancer survey (the SEER report [Ries et. al 2003]) found a greater increase in osteosarcomas in young males in fluoridated areas. However, Hoover, et al. (DHHS, 1991) from the National Cancer Institute downplayed these findings based on the fact that the cancer incidences were not related to the duration of exposure. In 1992 Cohn found an increase in osteosarcoma in fluoridated areas in NJ. In three counties he found nearly a seven fold incidence of osteosarcoma in young males in fluoridated towns compared to non-fluoridated ones. There was little difference in the rates for females. We would also note that the osteosarcomas found in the NTP study may not be the only cancers found in this study. Some cancers were removed in a controversial review process (Marcus, 1990).

EPA RESPONSE:

> “The possibility that fluoride might increase the cancer risk was raised in a series of reports which were considered by the 1993 NRC. Additionally, the York Review has conducted a more recent evaluation of the literature and found that the epidemiologic literature falls short of establishing a causal association of increased cancer and exposure to fluoride in humans. The National Toxicology Program (1990) considered their own results as equivocal evidence of carcinogenicity. There is no new information that convinces EPA that there is a need to depart from OPP's use of the current Agency MCLG/MCL in pesticide risk assessments at this time.”

We find it interesting that while EPA cites the WHO (2002) report elsewhere that they do not note here the WHO (2002) comment on the NTP (1990) cancer study, with respect to osteosarcoma:

> "Such a (dose-dependent) trend associated with the occurrence of a rare tumour in the tissue in which fluoride is known to accumulate cannot be casually dismissed."

It should be noted that the York Review (McDonugh et al. 2000) mentioned by the EPA above did not consider any animal or biochemical studies in their report, so while the epidemiological evidence that fluoride may cause osteosarcoma in young males is mixed, the possibility is highly plausible from a biological perspective. Biological plausibility becomes critically important when one is trying to inform a weight of evidence analysis on an issue which presents mixed animal and epidemiological results.

Osteosarcoma is highly plausible from a biological perspective because: 1) in young men bones are turning over rapidly; 2) the bone is the site where fluoride accumulates; 3) fluoride is known to stimulate bone growth; 4) unscheduled or accelerated growth in tissues can lead to cancer, especially if it is accompanied by genetic damage in the dividing cells and 5) there are mechanisms whereby fluoride is known to cause such genetic damage.

Caverzasio et al. (1998) have offered a very plausible mechanism for fluoride's ability to stimulate the proliferation of osteoblasts (bone cells), a phenomenon exploited in the treatment of patients with osteoporosis. They hypothesize that aluminum fluoride [AlF4]- activates the G-protein (Gi), which triggers a cascade mechanism involving phosphorylation of the tyrosine side chains of several cellular proteins including extracellular signal-regulating kinase (ERK). ERK, once excited, results in cellular proliferation. As the name implies, ERK is normally excited by extracellular growth factors. Fluoride short circuits these normal growth factors and thus may trigger cell division. In laboratory studies, Farley (1983) found that fluoride can cause osteoblast proliferation at serum fluoride levels (2 umol/L = 38 ppb fluoride) widely surpassed in fluoridated communities.

Whenever cells divide there is a risk that mutations may occur, and mutations in the sensitive spots (such as tumor suppressor genes) can lead to uncontrolled cell growth and then to cancer. This risk would be increased if the agent that stimulates cell division also causes damage to the genetic material. There is a growing body of evidence that fluoride does this.

In 1996, Mihashi and Tsutsui were able to demonstrate that fluoride caused chromosomal aberrations in a time and dose dependent manner in cultured cells derived from the vertebral bones of the same strain of rats (F344/N) used in the NTP rat-osteosarcoma study. Effects were observed at 4.3 ppm fluoride, a level which can be anticipated in key microenvironments in the bone in vivo. They argued that their results, "…demonstrate that NaF is clastogenic to rat vertebral body-derived cells, providing a mechanistic basis for NaF to induce osteosarcomas in NaF-treated rats."

Several other studies have demonstrated fluoride's ability to cause chromosomal aberrations, sister chromatid exchange (SCE), and other indicators of genetic damage in tissue cultures and in humans. For example Tsutsui et al. (1984) demonstrated that sodium fluoride induced "…morphological and neoplastic transformation, chromosome

aberrations, sister chromatid exchanges and unscheduled DNA synthesis…" in cultured Syrian hamster embryo cells.

Several research teams have demonstrated significantly higher SCE rates in the peripheral blood lymphocytes in persons living in areas of endemic fluorosis compared to those living in areas of low fluoride in the water - both in India and in China. These are very striking findings considering that in two of these studies the level in the endemic areas was not very high (1.56 -3.46 ppm and 1.95 - 2.2 ppm) i.e. levels below the EPA's current MCL of 4 ppm. Meng et al. (1995) have shown similar results for workers in a phosphate factory in China compared to other local residents not working in the factory.

When we combine all the findings discussed above, the issue of fluoride's possible connection to osteosarcoma cannot be dismissed lightly - especially with such a potentially serious outcome.

As for the possibility that fluoride may cause other types of cancer, it is regrettable that EPA did not respond to the discussion above on the downgrading of other cancers in the NTP (1990) rat study. As EPA has ignored this point we will add a few more pertinent details. Battelle Laboratories, the NTP contractor for the rat studies, initially reported numerous cancers (including oral cavity and liver), but these were later downgraded by a separate panel convened by the NTP which reviewed the tissue slides. Scientists at the EPA strongly objected to these downgradings - particularly since Dr. Melvin Reuber, who first discovered the rare type of liver tumor (hepatocholangiocarcinoma) reported in the NTP assay, reviewed the slide and stated that it was in fact a hepatocholangiocarcinoma (Marcus, 1990). Had this and the other tumors not been downgraded to non-cancerous neoplasms, fluoride would have been classified as a "probable human carcinogen". Because of the importance of the NTP's tumor slides regarding fluoride's potential as a carcinogen, and considering the controversy surrounding the NTP's interpretation of them, the Union that represents the scientists at EPA headquarters in Washington, DC has called for an independent review of these slides. Sadly EPA does not share the same concern for integrity on this issue as its own professional union.

Just in case there is any doubt in the matter, here is a listing of the studies that have found fluoride causes mutagenic and chromosomal damage:

Deleterious mutagenic effects (NaF) from NIOSH RTECS

Type of Test:mutation in microorganisms
Test System:salmonella typhimurium
Dose: 1 mg / plate
Reference: Cytology and Genetics. 16(6), 41, 1982

Type of Test:DNA repair
Test System:bacillus subtilis

Dose: 86 mg/L
Reference: WATRAG Water Research. 14,1613, 1980

Type of Test:sex chromosome loss and nondisjunction
Test System:oral
Dose: drosophila melanogaster
Reference: Fluoride. 6, 113, 1973

Type of Test:sex chromosome loss and nondisjunction
Test System:drosophila melanogaster
Dose: multiple routes
Reference: Hygiene in Populated Places. 15, 36, 1976.

Type of Test:unscheduled DNA synthesis
Test System:human fibroblast
Dose: 100 mg/L
Reference: Mutation Research. 139, 193, 1984

Type of Test:unscheduled DNA synthesis
Test System:hamster cells
Dose: 200 mg/L
Reference: Mutation Research. 140, 43, 1984

Type of Test:DNA inhibition
Test System:human fibrobalst
Dose: 100 mg/L
Reference: Mutation Research. 279, 109, 1992

Type of Test:cytogenetic analysis
Test System:human fibroblast
Dose: 20 mg/L
Reference: Mutation Research. 139, 193, 1984

Type of Test:cytogeic analysis
Test System:human lymphocyte
Dose: 20 mg/L
Reference: Mutagenesis. 2, 497, 1987.

Type of Test:cytogenic analysis
Test System:human cells
Dose: 952 umol/L
Reference: Toxicology In Vitro. 5, 353, 1991

Type of Test:mutation in mammalian somatic cells
Test System:human lymphocyte
Dose: 440 mg/L

Reference: Mutation Research. 196, 61, 1988

Type of Test: DNA damage
Test System: rat liver
Dose: 1 mmol/L
Reference: Mutation Research. 368, 59, 1996

Type of Test: unscheduled DNA synthesis
Test System: rat liver
Duration: 36 mg/L
Reference: Mutation Research. 172, 77, 1986

Type of Test: cytogenic analysis
Test System: rat bone marrow
Dose: 100 nmol/L
Reference: Mutation Research.

Type of Test: cytogenic analysis
Test System: rat cells
Dose: 500 umol/L
Reference: Mutation Research. 368, 7, 1996

Type of Test: micronucleus
Test System: mouse - oral
Dose: 40 mg/kg
Reference: Heredity. 8(5), 39, 1986.

Type of Test: micronucleus
Test System: mouse - intraperitoneal
Dose: 10 mg/kg
Reference: Caryologia. 40, 79, 1987

Type of Test: mutation
Test System: mouse lymphocyte
Dose: 300 ug/l
Reference: Mutation Research. 187, 1965, 1987

Type of Test: cytogenic analysis
Test System: mouse - intraperitoneal
Dose: 10 mg/kg
Reference: Caryologia. 40., 79, 1987

Type of Test: cytogenic analysis
Test System: mouse - oral
Dose: 1 ppm (3 weeks)
Reference: Fluoride. 15, 110, 1982

Type of Test:cytogenic analysis
Test System:mouse cells
Dose: 200 mg/L
Reference: Archives of Environmental Health. 29, 230, 1974

Type of Test:cytogenic analysis
Test System:mouse - subcutaneous
Dose: 40 mg/kg
Reference: Caryologia. 40, 79, 1987

Type of Test:sister chromatid exchange
Test System:mouse - intraperitoneal
Dose: 40 mg/kg
Reference: Heredity. 8(5), 39, 1986

Type of Test:mutation in mammalian somatic cells
Test System:mouse lymphocyte
Dose: 20 mg/L (16-hrs)
Reference: Mutagenesis. 1, 157, 1986

Type of Test:sperm morphology
Test System:mouse - intraperitoneal
Dose: 10 mg/kg (5-days)
Reference: Caryologia. 40, 79, 1987

Type of Test:micronucleus
Test System:hamster ovary
Dose: 1 mmol/L
Reference: Mutation Ressearch. 367, 99, 1996

Type of Test:morphological transformation
Test System:hamster embyro
Dose: 25 mg/L
Reference: Toxicology In Vitro. 2, 103, 1988

Type of Test:unscheduled DNA synthesis
Test System:hamster embyro
Dose: 10 mg/L
Reference: Cancer Research. 44, 938, 1984

Type of Test:DNA inhibition
Test System:human lung
Dose: 100 mg/L
Reference: Mutation Research. 279, 109, 1992

Type of Test:mutation test systems not otherwide specified
Test System:hamster lung
Dose: 100 mg/L
Reference: Mutation Research. 279, 109, 1992

Type of Test:cytogenic analysis
Test System:hamster embryo
Dose: 50 mg/L
Reference: Cancer Research. 44, 938, 1984

Type of Test:cytogenic analysis
Test System:hamster lung
Dose: 25 mg/L
Reference: Cytologia. 52, 889, 1987

Type of Test:cytogenic analysis
Test System:hamster ovary
Dose: 50 mg/L
Reference: Mutation Research. 223, 191, 1989

Type of Test:sister chromatid exchange
Test System:hamster embyro
Dose: 80 mg/L
Reference: Cancer Research. 44, 938, 1984

Type of Test:suster chromatid exchange
Test System:hamster ovary
Dose: 66,700 ug/l
Reference: NTP-TR-393, 1990

Type of Test:cytogenetic analysis
Test System:mammal domestic cells
Dose: 25 mg/L
Reference: Archives of Environmental Health. 29, 230, 1974

Type of Test:cytogenetic analysis
Test System:mammal - lung
Dose: 300 umol/L
Reference: Environmental Sciences Journal. 3, 94, 1983

Type of Test:cytogenetic analysis
Test System:cattle cells
Dose: 10 mg/L
Reference: Archives of Environmental Health. 29, 230, 1974

Type of Test:cytogenetic analysis

Test System:mammal lymphocyte
Dose: 4 mmol/L
Reference: Mutation Research. 301, 183, 1993

Type of Test:sister chromatid exchange
Test System:mammal - lung
Dose: 3 mmol/L
Reference: Environmental Sciences Journal. 3, 94, 1983

Indeed, in a review of the genetic toxicity of fluoride (Zeiger, et al. 1993) states that gene mutations in human cells were produced in the majority of cases -- and "the weight of the evidence leads to the conclusion that fluoride does result in increased chromosome aberrations".

Zeiger, et al. (1993). Genetic toxicity of fluoride. Environ Mol Mutagen; 21(4): 309-318.
