THE EFFECT OF THE FLUORIDE ION ON REPRODUCTIVE PARAMETERS AND AN ESTIMATE OF THE SAFE DAILY DOSE OF FLUORIDE TO PREVENT FEMALE INFERTILITY AND MISCARRIAGE, AND FOETAL NEUROTOXICITY

ABSTRACT: A paper in the current issue by Yousefi et al. examined data on the prevalence of fertility, infertility, and abortion (miscarriage), in the health records of 3,392 women, aged 10–49 yr, living in two regions of Poldasht county, Iran, with low and high drinking water fluoride ion (F) levels (means 1.90 and 8.10 mg/L, respectively) and found that those in the low F group (n=1,294), when compared to those in the high F group (n=2,098), were more fertile ($p<0.05$) and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$). A safe daily dose of F to prevent the adverse effects of infertility and abortion in humans of approximately 0.6 mg F/day was calculated from the findings of Yousefi et al. by taking conservative figures so as not to produce a falsely low safe dose [estimated daily F intake from drinking 1.5 L of water with 8 mg F/L = 12 mg F, estimated daily F intake from food = 5 mg, estimated total daily intake = 17 mg F, uncertainty factor to convert from a Lowest Observed Adverse Effect Level (LOAEL) to a No Observed Adverse Effect Level (NOAEL) = 3, uncertainty factor to allow for inter-individual variability =10, total uncertainty factors = 30, 17 ÷ 30 = 0.56, approximately 0.6 mg F/day or, assuming a body weight (bw) of 56 kg, 0.01 mg F/kg bw/day]. In conclusion, while a maternal dose of 0.6 mg F/day or 0.01 mg F/kg bw/day can be estimated to be protective of impaired fertility and abortion in humans, this level of intake is above the estimated maternal dose of 0.04 mg F/day, or 0.0007 mg F/kg bw/day, necessary to protect the foetus from neurotoxicity.

Keywords: Abortion; Fertility; Fluoride; Infertility; Miscarriage; Neurotoxicity; Safe daily dose of F.

A paper in the current issue by Yousefi et al. examined data on the prevalence of fertility, infertility, and abortion (miscarriage), in the health records of 3,392 women, aged 10–49 yr, living in two regions of Poldasht county, Iran, with low and high drinking water fluoride ion (F) levels (means 1.90 and 8.10 mg/L, respectively).[1] The women in the low F area (Daiankendi, Eshgabad, and Moradloo) numbered 1,294 and 2,098 women lived in the high F area (Sarisoo and Konikor). Women with known causes of infertility such as diabetes mellitus, obesity, smoking, and the consumption of alcohol, were excluded from the study. No statistically significant differences in the reproductive parameters were found between the low and high F regions when the women were considered by 5-year age groups, but, when the data were pooled and all the age groups were considered together in a group with ages 10–49 yr, those in the low F group were more fertile ($p<0.05$) and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$). This paper adds significantly to our knowledge of how the fluoride ion affects female reproductive parameters in humans.

The Committee on Fluoride in Drinking Water of the National Research Council, USA, reviewed a large number of reproductive and developmental studies in animals published since 1990 in the 2006 publication *Fluoride in drinking water: a scientific review of EPA's standards* and found the overall quality of the data base had improved significantly.[2a] High quality studies in laboratory animals over a drinking water F range of 0–250 mg/L indicated that adverse reproductive and developmental outcomes occurred only at very high concentrations. It is noted that it has been suggested that, compared to humans, rats might require water concentrations of F about five times larger to reach the same plasma concentration,[2b,3] that 12 week-old-rats have a renal clearance more than three times larger,[2b,4] and that rat extrarenal clearance was about twice as large.[2b,4] The Committee noted that a few studies of human populations have suggested that F might be associated with alterations in reproductive hormones, fertility, and Down's syndrome, but their design limitations made them of little value for risk evaluation.

The Committee commented that, in the 1994 ecological study by Freni[5] in 30 regions spread over nine states where the drinking water F was ≥3 mg/L, there was an association between decreasing total fertility rate and increasing fluoride concentrations in most regions. Freni found a significant negative association, based on population means rather than individual women, between the total fertility rate and the drinking water F level (p=0.0002–0.0004). The Committee considered that the overall study approach used by Freni had merit and could yield valuable new information if more attention was given to controlling for reproductive variables at the individual and group levels. Because Freni's study had design limitations, additional research was seen to be needed to substantiate whether an association existed between fertility and the drinking water F level. Two other research recommendations made by the Committee involved (i) a case-control study of the incidence of Down's syndrome in young women with differing levels of F exposure and (ii) carefully controlled studies of occupational exposure to F and the reproductive parameters found altered by Ortiz-Perez et al.[6] in 2003 involving 126 male workers in Mexico, aged 20–50 yr, exposed to high (3–27 mg/day) or low (2–13 mg/day) amounts of F. Ortiz-Perez et al. found a significant increase in FSH ($p<0.05$), a reduction of inhibin-B, free testosterone, and prolactin in serum ($p<0.05$), and a decreased sensitivity in the FSH response to inhibin-B ($p<0.05$) in the high F-exposed group compared to the low F-exposed group. The authors concluded that a F exposure of 3–27 mg/day induced a subclinical reproductive effect that could be explained by a F-induced toxic effect in both Sertoli cells and gonadotrophs.

Since the 2006 NRC review, Sun et al.[7] in 2009 studied sperm quality, sperm hyperactivation, and the gene expression of Catsper1 in adult male Kunming mice exposed to 150 mg sodium fluoride (NaF)/L for 7 weeks in their drinking water. Compared with the controls, the sperm quality and the proportion of hyperactivated sperm were significantly decreased in the mice treated with F. Likewise, the Catsper1 gene expression level was also significantly reduced in the treatment group. On the basis of these findings, they proposed that low Catsper1 gene expression in sperm may be associated with decreased sperm hyperactivation by NaF.

Also in 2009, Wang et al.[8] investigated the effects of NaF on sperm motility, oxidative stress, and apoptosis in the testes of male Wistar rats exposed to 1.0, 2.0, and 3.0 mg NaF/kg bw/day by intragastric gavage for 90 days. Sperm motility was significantly inhibited, especially at the lower F intake level. Significant increases in oxidative stress occurred with elevated malondialdehyde in the 1.0 mg NaF group and increased hydrogen peroxide in the 2.0 mg NaF group. Compared with the control group without NaF, the cell percentage in G0/G1 phase increased significantly, whereas the cell percentage in S phase decreased significantly. On the other hand, the percentage of cells in G2/M phase was similar to that of the control. In the 2.0 and 3.0 mg NaF groups, a significant increase in testicular cell apoptosis was observed. The authors concluded that, especially at a comparatively lower level of exposure, F exhibits toxic effects on reproductive function in the form of decreased sperm motility, enhanced oxidative stress, and increased apoptosis, although the latter does not appear to be directly connected with the increased level of oxidative stress.

Long et al.[9] reviewed F toxicity in the male reproductive system in 2009 and found that the most important consequences of high F exposure were: changes in the structure and functional behavior of spermatozoa, disruption of spermatogenesis, and disturbances of multiple hormone systems that impact male reproduction. The changes in spermatozoa resulted from oxidative damage, zinc deficiency, and disturbed signal transduction. There was evidence that F interfered with spermatogenesis by depressing levels of epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR), modifying G-protein signaling, diminishing levels of testosterone and its androgen receptor (AR), and disturbing levels of estradiol. Furthermore, F was also known to interfere with thyroid hormone metabolism, which directly and indirectly impacted not only spermatogenesis but also other reproductive functions. Although F appeared to exert its toxic effects in the male reproductive system through these pathways, the molecular details were seen to be still poorly understood.

Susheela[10] reported in 2010 that a programme emphasizing a greatly reduced intake of F and the inclusion of essential nutrients in the daily diet during pregnancy led to a striking increase in haemoglobin, an improved body mass index, fewer low birth weight babies, and reduced numbers of pre-term deliveries.

A further 2017 study by Sun et al.[11] broke new ground in our understanding of how F may adversely affect male fertility. Using iTRAQ-based comparative proteomics techniques to detect global changes in the protein profiles of testis in male mice after exposure to F in drinking water, the authors found F exposure may interfere with spermatogenesis, sperm motility, and the acrosome reaction. The suggested mechanisms involved adversely affecting ubiquitination, phosphorylation, retinoic acid synthesis, histone-protamine replacement, and the sperm acrosome membrane.

The above studies and others from the literature suggest that F may adversely affect both female and male fertility.[12,13] The dose at which this occurs is a relevant question. Impaired fertility has been reported *in vivo* in several animal species (rats, chinchillas, alligators, caimans, and horses) drinking water with approximately 1 mg F/L.[12] An approximation of a safe daily dose of F to prevent the adverse effects on infertility and abortion in humans can be calculated from the findings of Yousefi et al. using methodology based on that used by the United States Environmental Protection Agency (US EPA).[14] Taking conservative figures so as not to produce a falsely low safe dose, a safe daily dose of F would be approximately 0.6 mg F/day or 0.01 mg F/kg body weight (bw)/day [estimated daily intake from drinking 1.5 L of water with 8 mg F/L = 12 mg F, estimated daily F intake from food = 5 mg, estimated total daily intake = 17 mg F, uncertainty factor to convert from a Lowest Observed Adverse Effect Level (LOAEL) to a No Observed Adverse Effect Level (NOAEL) = 3, uncertainty factor to allow for inter-individual variability =10, total uncertainty factors 3×10 = 30, 17 ÷ (3×10) = 0.56, approximately 0.6 mg F/day or 0.01 mg F/kg body weight (bw)/day for a 56 kg adult].

The question then arises as to whether this dose of 0.6 mg F/day which is estimated to be protective of impaired fertility and abortion in humans would also protect the foetus from impairments of neural development. Bashash et al.[15] studied prenatal fluoride exposure and cognitive outcomes in Mexican children at 4 and 6–12 yr of age in a longitudinal study. Urine samples were taken from the mothers during pregnancy and from their children, when aged 6–12 yr old, and adjusted for urinary creatinine and specific gravity, respectively. Child intelligence was measured by Spanish versions of the General Cognitive Index (GCI) of the McCarthy Scales of Children's Abilities at age 4 yr and the full scale intelligence quotient (IQ) from the Wechsler Abbreviated Scale of Intelligence (WASI) at age 6–12 yr. By virtue of living in Mexico, the subjects were exposed to fluoridated salt at 250 ppm and varying degrees of naturally occurring F in drinking water, the range from the literature being 0.15–1.38 mg/L. The authors obtained complete data on 299 mother-child pairs of whom 287 and 211 had data for the GCI and IQ analyses, respectively. Maternal bone lead and blood mercury levels were measured but there was a lack of information about environmental neurotoxicants such as arsenic or about iodine in salt which could modify the associations between F and cognition. However, there was no evidence to suggest the subjects were exposed to significant levels of arsenic or other known neurotoxicants. The urinary F value for all of the mothers with complete data (n=299) was 0.90±0.35 mg/L (mean±SD) and for the children with available urine samples (n=211) it was 0.82±0.38 mg/L. For all 512 of the mothers studied, including those with incomplete data, the urinary F was 0.88±0.34 mg/L, range 0.02–2.36 mg/L, and 0.64, 0.82, and 1.02 mg/L for the 25th, 50th, and 75th percentiles, respectively. Using multivariate models, the authors found that an increase in the maternal urine F of 0.5 mg/L (approximately the interquartile range [IQR]) predicted 3.15 (95% CI= –5.42, –0.87) and 2.50 (95% CI –4.12, –0.59) lower offspring GCI and IQ scores, respectively. They concluded that higher prenatal F exposure, in the general range of exposures reported for other general population samples of pregnant women and non-pregnant adults, was associated with lower scores on tests of cognitive function in the offspring at age 4

yr and 6–12 yr. No clear, statistically significant association was present between the contemporaneous children's urinary fluoride at age 6–12 yr and IQ.

The authors found an increase in the maternal urine F of 0.5 mg/L during pregnancy was associated with a 2.50 IQ point decrease in the IQ of the children at age 6–12 yr. Thus, the 1.0 mg/L difference in the maternal urine F at the lowest part of the range of 0.02 mg/L and at the 75th percentile of 1.02 mg/L would be associated with a 5 point IQ decrease (2.50×1.0÷0.5=5). Similarly, the 0.62 mg/L difference in the maternal urine F at the lowest part of the range of 0.02 mg/L and at the 25th percentile of 0.64 mg/L would be associated with a 3.1 point IQ decrease (2.50×0.62÷0.5=3.1). In the same manner, a 0.285 mg/L difference in the maternal urine F at the lowest part of the range of 0.02 mg/L and at approximately the 12th percentile of 0.305 mg/L would be associated with a 1.4 point IQ decrease (2.50×0.285÷0.5=1.4).

Opydo-Szymacez and Borysewicz-Lewicka found the fasting morning urine F concentrations of 31 pregnant women, aged 22–34 yr, of 0.653 mg/L in the 28th week and 0.838 mg/L in the 33rd week were significantly ($p<0.01$) lower, by 50% and 44% respectively, than the mean value of 1.300 mg/L for healthy non-pregnant control women of similar ages, and noted that the difference may be explained by the incorporation of F into foetal hard tissues and accordingly decreased elimination of F in the urine.[16] Allowing for the urine F of a pregnant woman being 50% lower than in a non-pregnant woman, a maternal urine F of 0.305 mg/L would correspond to a non-pregnant person's urine F of 0.61 mg/L. A mean urine F of 0.61 mg/L was the average of three urine F levels of 0.57, (0.8 mg/day and assuming a 24-hr urine volume of 1.4L), 0.91 mg/L and 0.34 (0.481 mg/day and assuming a 24-hr urine volume of 1.4L) which was associated with a mean daily intake of 1.1 mg F/day, the average of 1.2,[2c,17] 0.93,[2d,18] and 1.190[2e,19] mg F/day, respectively.

If an intake of 1.1 mg F/day is regarded as the LOAEL, if an uncertainty factor of 3 is used to convert the LOAEL to a NOAEL, and if an uncertainty factor of 10 is used to allow for inter-individual variation, then a safe daily intake for pregnant women to give protection from foetal neurotoxicity would be 1.1 mg/day ÷ (3×10) = 0.04 mg F/day or, assuming a body weight of 56 kg, 0.0007 mg F/kg bw/day. This maternal exposure dose of 0.04 mg F/day, or 0.0007 mg F/kg bw/day, is 15 times less than the dose of 0.6 mg F/day or 0.01 mg F/kg bw/day which is estimated to be protective of impaired fertility and abortion in humans.

Because of the lack of a significant correlation between urinary F and IQ at age 6–12 yr and the presence of a significant negative relationship between the maternal urine F during pregnancy and the cognitive functioning tests at 4 yr and 6–12 yr, it appears that the intrauterine period is the time when the developing brain is most at risk for F neurotoxicity.

The mean consumption of municipal water for adults aged ≥20 yr, both direct and indirect involving beverages and foods that include water as an ingredient, has been estimated to be, in the USA, 1.1 L/day.[14] The F concentration of 1.1 L of water that would contain 0.04 mg F would be 0.036 mg/L or approximately 0.04 mg F/L. The maximum contaminant level goal (MCLG) is set by the US EPA under the 1974 Safe Water Drinking Act as a non-enforceable health goal based solely on possible health risks and is determined by the level of contaminants in drinking water at which no adverse health effects are likely to occur over a life time of exposure with an adequate margin of safety. For both lead and arsenic the MCLG has been set at zero. Theissen, a member of the committee that wrote the 2006 NRC report,[2f] was quoted recently as saying, "There is an increased risk of cognitive damage or other neurological damage with higher fluoride intake. The question is, is there a level below which it is safe? And I think probably not."[20] A similar editorial comment in *Fluoride* was made in 2011, "Thus, there is no threshold for F neurotoxicity in drinking water, and the only assuredly safe level is zero."[21]

F is not an essential trace element in humans or necessary for the development of healthy teeth and bones. The MCLG for fluoride should be zero, based on the results of the study by Bashash et al., the calculations above, and the other sources of F that humans have, such as

foods that may have high F levels naturally, e.g. tea, or from treatment with F-containing pesticides, e.g., grapes treated with cryolite, Na_3AlF_6, and dental products containing F that may be swallowed, e.g., fluoridated toothpaste. The currently recommended level of 0.7 mg F/L for community water systems[22] and the provision of fluoridated salt are no longer appropriate for preventing dental caries because they are likely to result in pregnant women having a F intake above the estimated safe daily intake of 0.04 mg/day. The quantity of water with 0.7 mg F/L that would contain 0.04 mg of F is 57 mL, only 5% of a daily intake of 1.1 L.

In conclusion, while a maternal dose of 0.6 mg F/day, or 0.01 mg F/kg bw/day, can be estimated to be protective of impaired fertility and abortion in humans, this level of intake is 15 times higher than the estimated safe maternal dose of 0.04 mg F/day, or 0.0007 mg F/kg bw/day, necessary to protect the foetus from neurotoxicity.

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