[Now published in full after the initial publication as an Epub ahead of print on June 4, 2020 at www.fluorideresearch.online/epub/files/085.pdf]

REVIEWS OF DEVELOPMENTAL FLUORIDE NEUROTOXICITY BY GRANDJEAN AND GUTH ET AL.

ABSTRACT: An updated review by Philippe Grandjean, published in *Environmental Health* on December 19, 2019, of developmental fluoride neurotoxicity concluded that recent epidemiological results support the notion that elevated fluoride intake during early development can result in IQ deficits that may be considerable. He noted that the recognition of neurotoxic risks is necessary when determining the safety of fluoride-contaminated drinking water and fluoride uses for preventive dentistry purposes. In contrast, another review by Guth et al., published online in *Archives of Toxicology* on May 8, 2020, concluded that fluoride was not a human developmental neurotoxicant at the current exposure levels in Europe where there is a mean fluoride intake in adults of 5–14 µg/kg bw/day in areas with a low drinking water fluoride level and of 30–40 µg/kg bw/day in areas with a high drinking water fluoride level. This editorial explores the reasons for the different conclusions being reached and finds that Guth et al. reached their conclusion by not considering the well-designed 2017 prospective cohort study by Basash et al. in sufficient detail to recognize that intrauterine exposure to fluoride is more important than exposure at 6–12 years in causing developmental fluoride neurotoxicity and by not considering the calculations made of the threshold for the drinking water fluoride level for the development of fluoride-induced neurotoxicity of approximately 0.2 mg/L, calculated by Hirzy et al. in 2016 and Grandjean in 2019. A drinking water fluoride level of 0.2 mg/L would result in a fluoride intake of 0.248 mg day or 3.5 µg/kg bw/day in a 70 kg adult with a water intake of 1.24 L/day which is less than the mean adult European intakes of 5–14 and 30–40 µg/kg bw/day in areas with low and high drinking water fluoride levels, respectively. The conclusions that can be drawn from the studies by Basash et al, Hirzy et al., and Grandjean, together with the prospective studies by Valdez Jiménez et al. and Green et al., are that intrauterine exposure to fluoride is more important than exposure during childhood at age 6–12 years in causing developmental fluoride neurotoxicity and that the threshold level for drinking water fluoride for the development of this effect is approximately 0.2 mg/L. A further 2019 study by Till et al. indicates that the developing brain may also be adversely affected by exposure to drinking water with approximately 0.6 mg/L of fluoride during infancy. Accordingly, exposure of pregnant women and infants to community water fluoridation, with water with 0.7 mg/L of fluoride, the level recommended in the USA for community water fluoridation, or with 0.6–0.8 mg/L the level recommended in the Republic of Ireland, or to the current exposure levels in Europe, where there is a mean fluoride intake in adults of 5–14 µg/kg bw/day in areas with a low drinking water fluoride level and of 30–40 µg/kg bw/day in areas with a high drinking water fluoride level, is unsafe, will lead to a fluoride intake that exceeds the threshold for developmental fluoride neurotoxicity of 0.2 mg/L or 3.5 µg/kg bw/day, and will result in the occurrence of developmental fluoride neurotoxicity. For these reasons, rather than accepting the reassurance of Guth et al., the editorial concurs with Grandjean's conclusion.

Keywords: Developmental fluoride neurotoxicity; Guth et al.;Grandjean; Review.

As commented on in a recent editorial,[1] an updated review by Philippe Grandjean, published in *Environmental Health* on December 19, 2019, of developmental fluoride neurotoxicity concluded that recent epidemiological results support the notion that elevated fluoride intake during early development can result in IQ deficits that may be considerable.[2] He noted that the recognition of neurotoxic risks is necessary when determining the safety of fluoride-contaminated drinking water and fluoride uses for preventive dentistry purposes.

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A contrasting conclusion was reached by Guth et al. in another review article published online in *Archives of Toxicology* on May 8, 2020.[3] Guth et al. concluded that the totality of the currently available scientific evidence did not support the presumption that fluoride should be assessed as a human developmental neurotoxicant at the current exposure levels in Europe, where there is a mean fluoride intake in adults of 5–14 µg/kg bw/day in areas with a low drinking water fluoride level and of 30–40 µg/kg bw/day in areas with a high drinking water fluoride level. This editorial will attempt to explore some of the possible reasons for the different conclusions being reached.

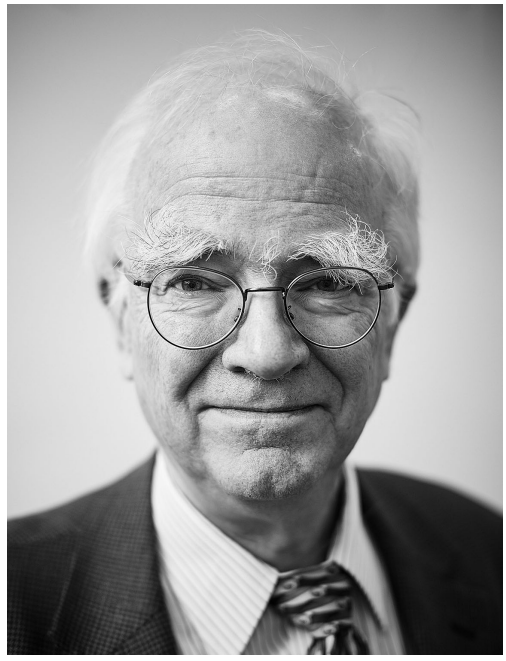

Photo courtesy of P Grandjean

Professor Philippe Grandjean, MD, DMSc (Copenhagen).

Guth et al. noted that recent, epidemiological studies, such as a 2014 review by Grandjean and Landrigan,[4] have suggested that fluoride is a human developmental neurotoxicant that reduces measures of intelligence in children, placing it into the same category as toxic metals (lead, methylmercury, and arsenic) and polychlorinated biphenyls. Guth et al. commented that, if true, this assessment would be highly relevant considering the widespread fluoridation of drinking water and the worldwide use of fluoride in oral hygiene products such as toothpaste. To gain a deeper understanding of these assertions, Guth et al. reviewed the levels of human exposure, as well as the results from animal experiments, particularly focusing on developmental toxicity, and the molecular mechanisms by which fluoride can cause adverse effects. Moreover, *in vitro* studies investigating fluoride in neuronal cells and precursor/stem cells were analyzed, and 23 epidemiological studies published since 2012 were considered. The results showed that the margin of exposure (MoE) between no observed adverse effect levels (NOAELs) in animal studies and the current adequate intake (AI) of fluoride (50 µg/kg bw/day) in humans ranged between 50 and 210, depending on the specific animal experiment used as a reference. Even for unusually high fluoride exposure levels, a MoE of at least ten was obtained. Furthermore, the concentrations of fluoride in human plasma were much lower than the fluoride concentrations causing effects in cell cultures. In contrast, 21 of 23 recent epidemiological studies reported an association between high fluoride exposure and reduced intelligence. Guth et al. considered that the discrepancy between the experimental and the epidemiological evidence may be reconciled by considering the deficiencies inherent in most of these epidemiological studies on a putative association between fluoride and intelligence, especially with respect to an adequate consideration of potential confounding factors, e.g., socioeconomic status, residence, breast feeding, low birth weight, maternal intelligence, and exposure to other neurotoxic chemicals.

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The Guth et al. review has 31 authors, from Germany (28), Austria (2), and Switzerland (1), is 41 pages long, and contains 168 references. In order to reach a better understanding of the potential associations between fluoride exposure and human intelligence, the authors conducted a PubMed literature search of epidemiological studies published between January 2012 and August 2019. Of the 23 epidemiological studies identified, 20 had a cross-sectional design,[5-24] one, by Bashash et al., was described as longitudinal[25] and two, by Broadbent et al. and Green et al., had a prospective cohort design.[26,27]

Guth et al. noted that, so far, almost all the studies investigating the effect of fluoride intake on intelligence were performed in relatively poor, rural communities, e.g., in China, Iran, and Mongolia, where the drinking water may contain comparatively high levels of fluoride ("exposed populations"), whereas the "reference populations" often had access to water that was fluoridated at the recommended level and that this constellation may lead to a confounding effect as the rural regions, with unusually high or unusually low fluoride levels in drinking water, may be associated with a less developed health-care system, as well as with a lower educational and socioeconomic status. They noted, furthermore, that in these regions the overall nutritional status and the intake of essential nutrients may be lower and the exposure to environmental contaminants which may affect intelligence, such as lead, cadmium, mercury, or manganese, may be higher. Conversely, the authors considered that the relatively rich communities with access to better education and/or higher socioeconomic status may be more likely to invest in having high-quality drinking water, e.g., to avoid fluoride concentrations above 1.5 mg/L to decrease the risk of dental fluorosis, and can afford the cost of the reduction of high fluoride concentrations through filtration. In addition, they observed that particularly low fluoride concentrations in drinking water can be rectified by fluoridation at adequate levels but that both measures require a relatively advanced public health-care system.

The Guth et al. review focused on the two prospective cohort studies published since 2012, by Broadbent et al.[26] and Green et al.,[27] which investigated the effect of fluoride exposure in drinking water resulting from community water fluoridation (CWF), i.e., in areas where water is fluoridated with a precise dose of fluoride as a public health prevention measure. They commented that, in contrast, most of the studies (21 of the 23 studies) investigated the effect of fluoride exposure in drinking water resulting from endemically occurring fluoride where the fluoride was naturally present at varying concentrations, with a range of 0.08–18.08 mg/L. Guth et al. reflected that cross-sectional and ecological studies do not allow the establishment of causal relationships and are not appropriate to ultimately evaluate the effect of chronic fluoride exposure on a parameter like human intelligence, but serve for the derivation of hypotheses. In contrast, prospective studies in which, over time, the cohorts are followed and the data relating to predetermined exposures and outcomes are collected, were considered appropriate for inferring causality.

Guth et al. noted that only 11 of the 23 studies published since 2012 performed a statistical adjustment for potential confounding factors and that in most of them the included confounders were incomplete. Twelve of 23 studies aimed to consider the influence of potential confounding factors by their study design, e.g., by comparing

populations with "similar characteristics", did not consider the influence of confounding factors at all, or did not comment on this fact.

The Guth et al. review considered that, although most of the epidemiological studies performed in rural areas reported an association of high fluoride exposure with lower intelligence, most of these studies were of low quality (e.g., insufficient control of confounding factors and no individual level exposure assessment) and were inadequately designed to prove or disprove hypotheses (cross-sectional). In contrast, the authors noted that the two available studies with a suitable study design (prospective cohort studies) conducted in non-endemic CWF areas that also appropriately considered confounding factors produced conflicting results. Broadbent et al. reported that no statistically significant differences in IQ due to fluoride exposure were observed, even after adjustment for potential confounding variables, including sex, socioeconomic status, breastfeeding, birth weight, and educational attainment (for adult IQ outcomes).[26] In the other prospective cohort study, Green et al. concluded that maternal exposure to higher levels of fluoride during pregnancy was associated with a significantly lower full-scale IQ scores in boys.[27] An increase of 1 mg/L of in the maternal urinary fluoride (MUF) was significantly associated with a 4.49 (95% CI –8.38 to –0.60) lower full scale intelligent quotient (FSIQ) score.[27] Girls showed a slight but non-significant increase in IQ scores (B = 2.40; 95% CI –2.53 to 7.33). A 1 mg higher daily intake of fluoride among pregnant women was significantly associated with a a 3.66 lower IQ score (95% CI –7.16 to –0.14) in boys and girls although the mean FSIQ was the same among children from the non-fluoridated (108.07) and fluoridated (108.21) areas.

Since the two available prospective studies led to different results, the authors systematically compared features that may explain the discrepancy noting that a limitation of both studies was the lack of IQ data of the mothers, because parental IQ is a strong confounder. They commented that an additional limitation of the Green et al. study was that the intelligence tests were performed only once between the age of 3 and 4 years and that the exact age of the children at the time point of the test had not been considered in the statistical analysis. This could be problematic, because the IQ of children changes strongly between 3 and 4 years and the Wechsler Preschool and Primary Scale of Intelligence Test (WPPSIIII) used in the study provides different sets of subtests for the 2:6–3:11 (years:months) age band and the 4:0–7:7 age band. In contrast, Broadbent et al. used a more robust measure of intelligence, the average of the IQ at ages 7, 9, 11, and 13 years. Broadbent et al. studied a birth cohort of 1,037 children (91% of eligible births) with a 95.4% retention after 38 years of prospective follow-up while Green et al. considered only 30.5% of the women in the Maternal Infant Research Environmental Chemicals (MIREC) program cohort. Bias may also have arisen in the study by Green et al. from the information on maternal urinary fluoride being absent in a relatively high fraction of the mothers of children of whom IQ was determined and the use of creatinine-adjusted urinary fluoride concentrations to account for urinary dilution which may cause an additional bias if a study participant suffered from renal problems influencing the IQ. Broadbent et al. studied the influence of possible confounding factors and obtained significant associations of socioeconomic status, breastfeeding, and low birth weight with the IQ which were then used to adjust the analysis of community water fluoridation with IQ. However,

limitations of the Broadbent et al. study were the lack of measurement of the individual water-intake level and the absence of consideration of the dietary fluoride. Green et al. did not consider breastfeeding and low birth weight as possible confounders but included some relevant confounders (city, socioeconomic status, maternal education, race/ethnicity, and prenatal secondhand smoke exposure). Green et al. also did not adjust for alcohol consumption and further dietary factors, other sources of fluoride exposure, the exact age of children at the time point of testing, and the children's postnatal fluoride exposure from sources other than drinking water, e.g., diet, fluoride dentifrice, and/or fluoride tablets.

After considering the limitations of the so far available epidemiological studies, Guth et al. found it was difficult to adequately interpret their findings since they presented heterogeneous results with a high risk of bias and that the only two studies with an appropriate study design, by Broadbent et al.[26] and Green et al.,[27] differed in important characteristics. Guth et al. concluded that the available epidemiological evidence does not provide sufficient arguments to raise concerns with regard to CWF in the range of 0.7–1.0 mg/L, and to justify the conclusion that fluoride is a human developmental neurotoxicant that should be categorized as similarly problematic as lead or methylmercury at current exposure levels.

It was not clear why the study by Bashash et al. was described by Guth et al. as longitudinal[25] while the two, by Broadbent et al.[26] and Green et al.,[27] were considered to have a prospective design. A longitudinal study is a research design that involves repeated observations of the same variables, e.g., people, over short or long periods of time, i.e., it uses longitudinal data. The subjects in the Broadbent et al. study, a cohort of 1,037 participants born in Dunedin during 1972–1973, who are followed in the Dunedin Multidisciplinary Health and Development Study, and have been used as an example of a longitudinal study.[28] A prospective cohort study is a longitudinal cohort study that follows over time a group of similar individuals (cohorts) who differ with respect to certain factors under study to determine how these factors affect rates of a certain outcome.[29] Bashash et al.[25] studied participants in the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) project and Green et al.[27] followed participants in the Maternal Infant Research Environmental Chemicals (MIREC) program. All three studies by Bashash et al.,[25] Broadbent et al.,[26] and Green et al.[27] can be regarded as being longitudinal prospective cohort studies and there does not appear to be valid reason to consider the Bashash et al. study as being inferior in design to the other two. To the contrary, it can be considered to have a stronger design than the Broadbent et al. study because individual data were collected on fluoride exposure (maternal urinary fluoride levels during pregnancy and urinary fluoride levels in their children at age 6–12 years) and to be stronger than the Green et al. study because the child intelligence levels were measured at both age 4 years (General Cognitive Index [GCI] of the McCarthy Scales of Children's Abilities) and at age 6–12 years (Full scale intelligence quotient [IQ] from the Weschler Abbreviated Scale of Intelligence [WASI]) for the reasons noted by Guth et al.[3] concerning IQ stability at ages 3-4 years and the changing subscales on the WPPSIIII between age 3 years 11 months and 4 years. Guth et al.[3] noted that Bashash et al. reported that higher fluoride exposure, in the general range found in 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 and 6–12 years. Bashash et al. found no clear, statistically significant association was present between the contemporaneous children's urinary fluoride at age 6–12 yr and IQ.[25] The findings of Bashash et al. pointed to intrauterine exposure during pregnancy being important in fluoride-induced developmental neurotoxicity rather than fluoride exposure at 6–12 years.

Thus the finding by Broadbent et al.[26] of the lack of a significant relationship between IQ and fluoride exposure in childhood, prior to age 5 years, is consistent with the findings of Bashash et al.[25] who did not find a relationship between IQ and fluoride exposure in childhood, at ages 6–12 years, although prenatal intrauterine fluoride exposure was significantly associated with impaired cognitive outcomes in children at ages 4, and 6–12 years. Thomas et al. added to Bashash's finding by reporting that *in utero* exposure to fluoride also had an adverse impact on offspring cognitive development at ages 1,2, and 3 years.[30]

The Broadbent et al. study has also been critiqued on the grounds that the estimated difference in the exposure to fluoride, from drinking water, food, toothpaste, beverages, and fluoride supplements, of less than 0.2 mg /day, between the CWF group and the non-CWF group (intakes of 1.36 and 1.19 mg/day, respectively) was so small that it was unlikely to lead to a detectable difference in IQ.[31]

The Guth et al. study did not comment on the two studies that used benchmark dose calculations to identify a likely threshold for fluoride neurotoxicity. Hirzy et al.[31] used this approach to generate benchmark results from a study by Xiang et al. of more than 500 children in China.[32] For a benchmark response (BMR) of 1 IQ point, the benchmark dose (lower confidence limit) (BMDL) was calculated to be a daily intake level of 0.27 mg/day.[31] Using the average water intake of 1.24 L/day in non-pregnant women, this BMDL of 0.27 mg/day corresponds to a water concentration of 0.22 mg/L.[31] The report did not provide data for urinary fluoride concentrations. Although the Hirzy et al.[31] paper was published in 2016 it would not have been detected by Guth et al. when searching the PubMed database for epidemiological studies published between January 2012 and August 2019 with the key words in the title/abstract including "fluoride" and "IQ" or "intelligence quotient" as *Fluoride* is not covered by PubMed.[33] Guth et al. also checked the reference lists of the included studies for further trials and this may have helped detect the six studies published in *Fluoride* that were included in their review but did not reveal the existence of the Hirzy et al. paper.

The other benchmark dose calculation to identify a likely threshold for fluoride neurotoxicity by Grandjean was published in *Environmental Health* on December 19, 2019 after the PubMed search period used by Guth et al. of January 2012 and August 2019 but before their paper was submitted for publication on January 27, 2020. Grandjean[2] used the regression coefficients and their standard deviations, as provided in the published reports of the Mexican and Canadian studies by Bashash et al.[25] and Green et al.,[27] respectively, to estimate the tentative bench mark dose (BMD) values. Assuming linearity and Gaussian distributions, he calculated the results for these two prospective studies with the maternal urinary fluoride concentration as the exposure parameter in regard to the cognitive function measures (both boys and girls). Overall, the BMDL results appeared to be in agreement. The Canadian children had lower

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prenatal exposures than the Mexican study subjects, and along with the apparent lack of fluoride effects in girls, the BMD results are higher than in the ELEMENT study, although the greater uncertainty results in a fairly low BMDL. The results suggest a BMDL of about 0.2 mg/L or below, a level that is similar to the result of 0.22 mg/L calculated from the Xiang et al. study in China[31,32] and clearly below the commonly occurring exposure levels, even in communities with drinking water fluoridation. Although Guth et al. commented on the 2014 Grandjean and Landrigan article[4] in *Lancet Neurology* on neurobehavioural effects of developmental toxicity, it is unfortunate that the timing of Grandjean's 2019 publication[2] did not allow them to address the issues raised by Grandjean in 2019, including the benchmark dose calculations.

Although Guth et al. conclude their review by stating that further high quality prospective epidemiological studies are required that adequately control for any cofounding factors, they have not given attention to the high quality studies of Bashash et al.[25] and Xiang et al.[32] which allow for the likely threshold for fluoride neurotoxicity to be estimated by using benchmark dose calculations. As noted, although the study by Xiang et al.[32] was cross-sectional rather than being prospective it used individual data. The fluoride levels in the drinking water in the high fluoride village Wamiao were able to be stratified into five groups and the confounding factors allowed for included co-exposures to lead, arsenic, and iodine, family income, and parental education levels.

The conclusion of the 2020 review by Guth et al. is that the totality of the currently available scientific evidence does not support the presumption that fluoride should be assessed as a human development neurotoxicant at the current exposure levels in Europe. They estimate the average exposure in adults in European areas with a low fluoride level in drinking water to be in the range of 5–14 µg/kg bw/day and for areas with high fluoride in drinking water to be in the range of approximately 30–40 µg/kg bw/day with a maximum of 63 µg/kg bw/day. The authors note that the levels for the mean intake of fluoride from food, water, beverages are generally below the adequate intake (AI) level of 50 µg/kg bw/day which is recommended for caries protection for all age groups and particularly for children.

This contrasts with Grandjean's conclusion in his 2019 review where he found that there is little doubt that developmental neurotoxicity is a serious risk associated with elevated fluoride exposure, whether due to community water fluoridation, natural fluoride release from soil minerals, or tea consumption, especially when the exposure occurs during early development. He found the threshold for developmental fluoride toxicity from fluoride in drinking water, the BMDL, to be approximately 0.2 mg/L which, with an average water intake of 1.24 L/day and an average body weight of 70 kg, corresponds to an intake of 3.5 µg/kg bw/day

Guth et al. formed their conclusion after reviewing the levels of human exposure, the results from animal experiments, particularly focusing on developmental toxicity, and the molecular mechanisms by which fluoride can cause adverse effects, *in vitro* studies of the effect of fluoride in neuronal cells and precursor/stem cells, and 23 epidemiological studies published since 2012, and found that the margin of exposure (MoE) between no observed adverse effect levels (NOAELs) in the animal studies and the current adequate intake (AI) of fluoride (50 µg/kg/day) in humans ranged

between 50 and 210, depending on the specific animal experiment used as reference. Even for unusually high fluoride exposure levels, an MoE of at least ten was obtained. Furthermore, the concentrations of fluoride in human plasma were much lower than the fluoride concentrations, causing effects in cell cultures. They attributed the association between high fluoride exposure and reduced intelligence found in 21 of the 23 recent epidemiological studies to deficiencies in the studies, especially with respect to the consideration of potential confounding factors, e.g., socioeconomic status, residence, breast feeding, low birth weight, maternal intelligence, and exposure to other neurotoxic chemicals.

By only looking in the PubMed database for studies for studies published between January 2012 and August 2019, and considering the reference lists in the included studies, the authors omitted consideration of the high quality cross-sectional study from 2003 by Xiang et al.[32] As noted, although the study was cross-sectional rather than prospective, it used individual data for the water fluoride and IQ measurements and the communities studied in the villages of Wamiao and Xinhuai had stable populations so that the fluoride exposure at the ages of 8–13 years reflected the levels of intrauterine fluoride exposure during pregnancy. The 122 children in Wamiao village were stratified into five groups according to the level of fluoride in their drinking water (<1.0, 1.0–1.9, 2.0–2.9, 3.0–3.9, and >3.9 mg/L) while the 159 children in the control village of Xinhuai had a level of 0.18–0.76. In addition, the confounding factors allowed for included co-exposures to lead, arsenic, and iodine, family income, and parental education levels.

The two prospective studies by Broadbent et al.[26] and Green et al.[27] with better designs that did consider possible confounding factors were looked at closely by Guth et al.[3] but gave conflicting results. Based on the totality of the evidence, the authors considered that fluoride should not be considered as a human developmental neurotoxicant at the current exposure levels in European countries and called for more research including sufficiently powered high-quality animal studies and high-quality prospective epidemiological studies.

Grandjean's review[2] updated the conclusions from his 2012 meta-analysis of cross-sectional studies of intellectual deficits associated with elevated fluoride exposure.[34] He noted subsequent epidemiological studies have strengthened the links to deficits in cognitive functions with several of them providing individual exposure levels, although most of the new studies were cross-sectional and focused on populations with fluoride exposures higher than those typically provided by fluoridated water supplies. However, prospective studies from the most recent years document that adverse effects on brain development happen at the elevated exposure levels that occur widely in North America and elsewhere in the world, in particular in communities supplied with fluoridated drinking water. His assessment was that these new prospective studies are of very high quality and, given the wealth of supporting human studies and biological plausibility, leave little doubt that developmental neurotoxicity is a serious risk associated with elevated fluoride exposure, especially when this occurs during early brain development.

Grandjean singled out three prospective studies, two in 2017 from Mexico by Valdez Jiménez et al.[35] and Bashash et al.[25] and one in 2018 from Canada by Green et al.,[27] which were seen to provide more robust evidence. The three studies all found

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that intrauterine fluoride exposure could result in fluoride-induced neurotoxicity. Grandjean used the regression coefficients and their standard deviations, from the studies by Bashash et al.[25] and Green et al.,[27] respectively, to estimate the tentative bench mark dose (BMD) values. Assuming linearity and Gaussian distributions, he calculated the results for these two prospective studies with the maternal urinary fluoride concentration as the exposure parameter in regard to the cognitive function measures (both boys and girls). Overall, the BMDL results appeared to be in agreement. The Canadian children had lower prenatal exposures than the Mexican study subjects, and along with the apparent lack of fluoride effects in girls, the BMD results are higher than in the ELEMENT study, although the greater uncertainty results in a fairly low BMDL. The results suggest a BMDL of about 0.2 mg/L or below, a level that is similar to the result of 0.22 mg/L calculated from the Xiang et al. study in China[31,32] and clearly below commonly occurring exposure levels, even in communities with drinking water fluoridation.

Guth et al. did not consider the Valdez Jiménez et al.[35] study in their review and did not pay particular attention to the Bashash et al.[25] study which was categorized as being longitudinal rather than being a prospective cohort. The Green et al.[27] study was looked at in detail but its results conflicted with the results of the study by Broadbent et al.[26] and both studies had some design deficiencies so that the discrepant results did not allow support for the presumption that fluoride was a human developmental neurotoxicant. No attempt was made to calculate the threshold for fluoride neurotoxicity by using the standard benchmark dose method and the two studies which had considered this were not discussed.

The key conclusion that can be drawn from the prospective studies by Valdez Jiménez et al.,[35] Bashash et al,[25] and Green et al.[27] is that timing is important in the development of fluoride-induced developmental fluoride neurotoxicity.[36] Exposure to the fluoride ion can, in a sufficient dose, induce neurotoxicity at any age, in both adults and children, but for fluoride-induced neurotoxicity to occur in the developing brain in response to exposure to low doses of fluoride, the timing of the exposure is of importance. The evidence from studies by Valdez Jiménez et al.,[35] Bashash et al,[25] and Green et al.[27] indicates that the developing brain is most sensitive to fluoride-induced neurotoxicity during the intrauterine period. Exposure to a low dose of fluoride later in childhood, at ages approximately 6–13 years, may or may not be associated with a reduction in IQ or school performance and may reflect the degree to which later childhood exposure parallels intrauterine exposure. In stable societies with a single source of fluoride, such as the water supply, and no fluoride pollution from burning coal or other industrial sources, e.g., the villages of Wamiao and Xinhuai in rural China studied by Xiang et al.,[32] a higher correlation may be present between intrauterine exposure and exposure later in childhood than in societies where multiple fluoride sources are present such as industrial sources, foods high in fluoride, fluoridated salt, and fluoridated toothpaste, e.g., Mexico City studied by Bashash et al.[25] and Thomas et al.[30] The findings of the 2019 by Soto-Barreras et al.[37] are also consistent with this interpretation of the data. The Soto-Barreras et al. study was published in *Fluoride* in July 2019 within the time frame of January 2012 to August 2019 used by Guth et al. in their PubMed search but not included by them because of the lack of PubMed coverage of *Fluoride*. In the Soto-Barreras et al.

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study,[37] a cross-sectional examination of 161 children in Chichuahua, Mexico, from 9 to 10 years of age, the concentration of fluoride in drinking water and urine was analyzed individually and the intellectual ability of children was evaluated through the Raven's Colored Progressive Matrices. In addition, the variables of diet, oral hygiene, body mass index, and socioeconomic status were included. No relationship was found between intellectual ability and the fluoride exposure variables such as, dental fluorosis, levels of fluoride in drinking water and urine, and exposure dose. In the three studies of Mexican children, by Valdez Jiménez et al.,[35] Bashash et al,[25] and Soto-Barreras et al.,[37] fluoride-induced neurotoxicity was significantly related to intrauterine fluoride exposure but not to exposure later in childhood at ages 6–12 years and 9–10 years.

Grandjean[2] considered that while evidence on the neurotoxic impact of early postnatal exposure remains limited, other neurotoxicity evidence suggests that adverse effects are highly plausible. He noted that the 2006 National Research Council of the National Academies (NRC) review *Fluoride in drinking water: a scientific review of EPA's standards* described research on laboratory animals which confirmed that elevated fluoride exposure was toxic to the brain and nerve cells.[38] Grandjean observed that the evidence today is substantially more robust.[2] The 2016 National Toxicology Program (NTP) review placed more confidence in fluoride impairing learning in adult animals due to fewer experimental studies being available on developmental exposure.[39] Grandjean noted that even although not all the studies were in agreement, perhaps due to species or strain differences in vulnerability, fluoride was known to pass the placental barrier and to reach the brain, and that the animal studies confirmed the importance of the prenatal period for fluoride-induced neurotoxicity.[2] He commented that toxicant exposures in early life can have much more serious consequences than exposures occurring later in life, and the developing brain is known to be particularly vulnerable.[2] Thus, the vulnerability of early brain development supported the notion that fluoride neurotoxicity during early life is a hazard of public health concern.

Two of the reasons for Guth et al.[3] reaching a different conclusion to Grandjean[2] are that (i) they did not consider the well-designed 2017 prospective cohort study by Basash et al.[25] in sufficient detail to recognize that intrauterine exposure to fluoride is more important than exposure at 6–12 years in causing developmental fluoride neurotoxicity and (ii) they did not consider the calculations made of the threshold for the drinking water fluoride level for the development of fluoride-induced neurotoxicity of approximately 0.2 mg/L, calculated by Hirzy et al. in 2016 and Grandjean in 2019. A drinking water fluoride level of 0.2 mg/L would result in a fluoride intake of 0.248 mg day or 3.5 µg/kg bw/day in a 70 kg adult with a water intake of 1.24 L/day which is less than the mean adult Euoropean intakes of 5–14 and 30–40 µg/kg bw/day in areas with low and high drinking water fluoride levels, respectively.

In the section of the Guth et al. review dealing with basics of fluoride toxicity the mechanisms of action are discussed. The authors note that fluoride interacts with proteins, particularly enzymes, and that although it usually inhibits enzyme activity at concentrations in the millimolar range, cell proliferation may be stimulated at concentrations in the micromolar range. Seven of the mechanisms by which fluoride

affects cell functions are listed: (i) the generation of superoxide anions; (ii) mitochondrial toxicity, e.g., opening of the transition pore; (iii) release of cytochrome c from mitochondria and the induction of apoptosis; (iv) inhibition of migration, e.g., of embryonic neurons and sperm; (v) increased endoplasmic reticulum stress in ameloblasts, the cell type responsible for enamel formation; (vi) increased expression of inflammatory factors, such as NF-kappaB and IL-8; and (vii) the modified release of the neurotransmitters acetylcholine and gamma-aminobutyric acid. Missing from the list was any mention of how fluoride, in the form of an aluminofluoride complex (AlF_x), is a phosphate group analogue which is able to mimic thyroid stimulating hormone (TSH) by switching on its associated G protein and it is suggested that the consequent overproduction of the second messenger cAMP leads to a feedback mechanism resulting in a desensitization of the TSH receptor and ultimately to a reduced activity of the thyroid gland.[40-42] The complexes formed by fluoride with metals such as aluminum and beryllium which mimic phosphate, such as AlF_4^- and $BeF_3^-{\bullet}H_2O$, have either positive or negative effects on a variety of enzymes and regulatory phosphatases.[43-45] In whole-cell systems, the intracellular effects of extracellular Al_3^+ and fluoride are often biphasic being stimulatory in low doses and inhibitory at high doses.[44] At low doses, biological systems display an overcompensation response which results in the apparent low-dose stimulation toxicity.[44] At higher doses with greater toxicity, the system often displays a more limited capacity for a compensatory response which is usually insufficient to return to the control levels.[44] Fluoride may also stimulate heterotrimeric G proteins in an Al_3^+- or Al_4^+-independent manner,[46] possibly by acting as a phosphatase inhibitor.[47] Hydrogen fluoride, HF, is a weak acid and can act as a transmembrane proton conductor and de-energize the cell membrane was discharging ΔpH.[43] Fluoride inhibits enzymes containing a metal, such as cytochrome oxidase containing Fe, enzymes that need a metal ion for activity, such as enolase which requires Mg^{2+}, and enzymes not containing or needing a metal ion for activity such as acetylcholinesterase where toxicity may follow the breaking up of existing hydrogen bonds and the formation of new ones.[48] Fluoride may stabilize activated receptors[49] and threaten microtubule stability.[50]

Grandjean[2] notes that although the adverse outcome pathway leading to developmental fluoride neurotoxicity is unclear, several epidemiological studies suggest that thyroid dysfunction is a relevant risk at elevated fluoride exposures.[51-55] Thus, studies in children have reported deficient thyroid functions, including elevated TSH (thyroid stimulating hormone) with elevated fluoride exposure, and one study linked elevated fluoride exposure to both thyroid dysfunction and IQ deficits.[56] In Canada, elevated urinary fluoride was associated with increased TSH among iodine-deficient adults, though not in the general population, after exclusion of those with known thyroid disease.[57] In England, the diagnosis of hypothyroidism was nearly twice as frequent in medical practices located in a fully fluoridated area, as compared to non-fluoridated areas.[58] These findings are highly relevant to the neurotoxicity concerns, as thyroid hormones are crucial for optimal brain development.[59,60]

The conclusions that can be drawn from the studies by Basash et al,[25] Hirzy et al.,[31] and Grandjean,[2] together with the prospective studies by Valdez Jiménez et al.[35] and Green et al.,[27] are that intrauterine exposure to fluoride is more important than

exposure during childhood at age 6–12 years in causing developmental fluoride neurotoxicity and that the threshold level for drinking water fluoride for the development of this effect is approximately 0.2 mg/L.

A further paper by Till et al.,[61] on the MIREC cohort reported on by Green et al,.[27] published online on November 16, 2019, indicates that the developing brain may also be adversely affected by fluoride exposure during infancy. Till et al.[61] found that 38% of mother-child dyads lived in fluoridated communities.(mean water fluoride concentration for the group breast-fed for more than 6 months and for the formula-fed groups 0.58 and 0.59 mg/L, respectively) rather than in non-fluoridated communities (mean water fluoride concentration 0.13 mg/l) and that an increase of 0.5 mg/L in water fluoride concentration (approximately equaling the difference between the fluoridated and non-fluoridated regions) corresponded to a 9.3- and 6.2-point decrement in the Performance IQ, measured on the WPPSIIII between age 3.0 and 4.0 years, in formula-fed and breast-fed children, respectively. The association between water fluoride concentration and Performance IQ remained significant after controlling for fetal fluoride exposure. A 0.5 mg increase in fluoride intake from infant formula corresponded to an 8.8-point decrement in Performance IQ and this association remained significant after controlling for fetal fluoride exposure. The authors concluded that exposure to increasing levels of fluoride in tap water was associated with diminished non-verbal intellectual abilities and that the effect was more pronounced among formula-fed children. The authors stated that in the absence of any benefit from fluoride consumption in the first six months it is prudent to limit fluoride exposure by using non-fluoridated water or water with a lower fluoride content as a formula diluent. Fluoride is considered to have predominantly topical benefit on teeth but this can not be exerted before the teeth have erupted which is at the age of approximately 6 months. Further to the findings by Valdez Jiménez et al.[35], Basash et al,[25], and Green et al.,[27] that intrauterine exposure to fluoride is more important than exposure during childhood at age 6–12 years in causing development fluoride neurotoxicity, the paper by Till et al.[61] indicates that the developing brain may be adversely affected by fluoride exposure during infancy.

Guth et al. considered that only two studies on fluoride-induced neurotoxicity were of high quality, those of Broadbent et al.[26] and Green et al.,[27] and as they produced conflicting results about whether or not fluoride was a neurotoxicant, the available evidence did not justify the rejection of the null hypothesis that fluoride was not a human developmental neurotoxicant at the current exposure levels in Europe, However, if a more specific null hypothesis had been examined, that fluoride exposure *in utero* and in infancy was not a human developmental neurotoxicant at the current exposure levels in Europe, then the results of the studies of Broadbent et al. and Green et al. could be seen as being in agreement and allowed the rejection of the null hypothesis. Apart from the issue of the low power of the Broadbent et al. study, with the difference in the fluoride intake between the CWF and the non-CWF groups being less than 0.2 mg /day, the study looked at fluoride intake during a wider range of childhood, prior to the age 5 years, rather than at fluoride exposure during the narrower period of *in utero* and during the first year (infancy).

Rather than accepting the reassurance of Guth et al., I concur with Grandjean's conclusion that there is little doubt that developmental neurotoxicity is a serious risk

associated with elevated fluoride exposure, whether due to community water fluoridation, natural fluoride release from soil minerals, or tea consumption, especially when the exposure occurs during early development. Exposure of pregnant women and infants to community water fluoridation, with water with 0.7 mg/L of fluoride, the level recommended in the USA for community water fluoridation,[62] or with 0.6–0.8 mg/L the level recommended in the Republic of Ireland,[63] or to the current exposure levels in Europe,[3] where there is a mean fluoride intake in adults of 5–14 µg/kg bw/day in areas with a low drinking water fluoride level and of 30–40 µg/kg bw/day in areas with a high drinking water fluoride level, is: (i) unsafe, (ii) will lead to a fluoride intake that exceeds the threshold for developmental fluoride neurotoxicity of 0.2 mg/L or 3.5 µg/kg bw/day, and (iii) will result in the occurrence of developmental fluoride neurotoxicity. In Grandjean's view, given that developmental neurotoxicity is considered to cause permanent adverse effects, the next generation's brain health presents a crucial issue in the risk-benefit assessment for fluoride exposure.[2]

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