FLUORIDE- AND ELECTROMAGNETIC RADIATION-INDUCED GENOTOXICITY AND IMPAIRED MELATONIN SECRETION

SUMMARY: Rao and Thakur have shown that the antioxidants melatonin and alma (*Emblica officinales*, Indian gooseberry) are effective, both individually and in combination, against fluoride-induced genotoxicity in human peripheral blood lymphocyte cells, which was first described in humans in 1994. Some animal and human work also suggests that fluoride (F) can impair the defensive response to genotoxicity by being deposited in high concentrations in the pineal gland and, through an enzyme-inhibiting action, reducing the secretion of melatonin, a powerful antioxidant able to eliminate free radicals and protect DNA. In having the capacity to be both genotoxic and impair melatonin secretion, F is similar to electromagnetic radiation, at power line frequencies and above, and both have very low or zero thresholds for causing toxicity. In view of the seriousness of neoplasia, the effect of fluoride on melatonin secretion warrants further research.

Keywords: Alma; Electromagnetic radiation and genotoxicity; Electromagnetic radiation and melatonin; Fluoride and genotoxicity; Fluoride and melatonin; Melatonin; Pineal inhibition by electromagnetic radiation; Pineal inhibition by fluoride.

In this issue Rao and Thakur (pp. 128-34) show that the antioxidants melatonin (N-acetyl-5-methoxytryptamine) and alma (*Emblica officinales*, Indian gooseberry) are effective, both individually and in combination, against fluoride-induced genotoxicity in human peripheral blood lymphocyte cells. The study follows several previous studies[1-3] since 2006 on the ameliorative effects of melatonin on fluoride-induced toxicity from Professor Mandava V Rao's group at Gujarat University, Ahmedabad, where the genotoxic effects for fluoride (F) in drinking water were first described in 1994 by Sheth, Multani, and Chinoy.[4] Professor Niloufer Chinoy's team found increased sister chromatid exchanges (SCEs) in persons exposed to F in the endemic areas of North Gujaret (F^- 1.95–2.2 mg/L or ppm) compared to those living in Ahmedabad (F^- 0.6–1.0 mg/L).

Rao and Thakur note that exposure to radioactive and genotoxic agents may affect the cell cycle and increase the frequency of SCEs, average generation time, and population doubling time. They found that F can bind to Ran protein, which is involved in the arrangement of microtubules and regulates nuclear cytoplasmic transport during the three phases of the interphase part of the cell cycle: G1 (cells increase their size), S (DNA replication occurs), and G2 (significant protein synthesis occurs, mainly for the production of microtubules).[5-6] SCEs occur during DNA replication as cells pass to S phase, and F affects the cell cycle, cell membrane, and protein leading to an increase in SCE frequency and a decreased cell cycle proliferative index. Several studies have linked F exposure to neoplasia including a 2006 report by Bassin, Wypij, Davis, and Mittleman, which found, in males, an association between F exposure in drinking water during childhood and the incidence of osteosarcoma before the age of 20.[7-12]

Rao and Thakur record that melatonin, secreted from the pineal gland, along with its by-products, is an extremely powerful antioxidant that is able to eliminate free radicals, such as reactive oxygen species, and has a particular role in

protecting DNA. Reduced melatonin secretion has been linked to DNA strand breaks, chromosome aberrations, and impaired immune system competence.[13,14]

Thus genotoxicity may result from the direct action of a toxic agent or indirectly through the toxic agent reducing the secretion of the free radical scavenger melatonin. In addition to the references above indicating a direct toxic action by F,[1-4,6] there is a literature reporting that F may reduce melatonin secretion. Luke found that prepubescent Mongolian gerbils (*Meriones unguiculatus*) fed a high-F diet had significantly lower pineal melatonin production than those fed a low-F diet, and sexual maturity occurred earlier in the females.[15,16] Melatonin activates cAMP-sensitive gene expression in the pars tuberalis of the anterior pituitary gland by the sensitization of adenylyl cyclase, thus synchronizing the suprachiasmatic nucleus of the hypothalamus and clock-controlled genes in the peripheral tissue which control the onset of puberty.[17,18] She noted that the possibility of a species difference between humans and gerbils did not allow the extrapolation of the gerbil data to humans but expressed some concern about the possible implications of the results of the animal studies. She observed that F was now being introduced to children to protect against dental caries at a much earlier stage of human development than had ever occurred before. If the resulting increased plasma-F levels have caused a decline in the levels of circulating melatonin during early human development, significant physiological consequences may have already occurred.

Luke found that the range for the pineal F content in eleven human cadaver pineal glands, from persons aged 72–100 yr, was 14–875 mg/kg with a mean of 296±257 mg/kg (wet weight), higher than that in corresponding muscle, 0.5±0.4 mg/kg (wet weight), and that it was directly correlated to the pineal calcium content: $r=0.73$, $p<0.2$.[15,19] The pineal is both a soft and a mineralizing tissue, and the mean F concentration found in the pineal calcification was equivalent to that in severely fluorosed bone and more than four times higher than in corresponding bone ash, i.e., 8,900±7,700 vs. 2,040±1,100 mg/kg, respectively. The calcification in two of the eleven pineal glands contained extremely high levels of F: 21,800 and 20,500 mg/kg. She considered that pinealocytes may not function normally in close proximity to high concentrations of F, which might affect the enzymatic conversion of tryptophan to melatonin or the synthesis of melatonin precursors, (e.g., serotonin), or other pineal products, (e.g., 5-methoxytryptamine).[15,20]

Although no studies are available that specifically address the effect of F exposure on pineal function or melatonin production in humans, two studies examined the age of onset of menstruation (age of menarche) in girls in fluoridated areas.[17] Schlesinger, Overton, Chase, and Cantwell found that this was 12 yr in fluoridated Newburgh, New York, (F^- 1.2 mg/L) compared to 12 yr 5 mo, in nonfluoridated Kingston ("essentially fluoride-free").[21] The result was not significant but, as it was a 10-year follow-up, the girls had not been exposed to F over their entire lives.[17] Some had been only exposed to F for only a few years, e.g., from age 8–9 yr and those girls in Newburgh, who had been exposed to fluoridated water since birth or before, had not reached menarche by the time of

the study. In Hungary, Farkas, Fazekas, and Szekeres found no difference in the menarcheal age of 12.779 yr in naturally-fluoridated Kunszentmárton (F^- 1.09 mg/L) and the age 12.79 yr in low-F Kiskunmajsa (F^- 0.17 mg/L).[22] However, the study showed that more postmenarcheal girls were present at younger ages in the high-F town than in the low-F town, although the reported median ages were the same.[17] Of those reporting having reached menarche by the time of the study (159 in Kunszentmárton and 270 in Kiskunmajsa), the youngest were 10 yr (1 girl), 11 (2 girls), and 11.5 (6 girls) in Kunszentmárton (8.0% of the total in the 10–11.5 age groups, 5.7% of all postmenarcheal girls in the high-F town) and 11.5 (5 girls) in Kiskunmajsa (4.7% of the total in the 10–11.5 age groups, 1.9% of all postmenarcheal girls in the low-F town). The F in the water in the two Hungarian towns was present naturally,[23] and Sauerheber has noted that natural fluorides accompanied by Group II cations such as calcium and magnesium have less chemical activity over a broad range of cation concentrations, even in the absence of precipitation, compared to when F is accompanied in solution with Group I metal cations, such as sodium or potassium.[24] He notes that sodium fluoride was added to the public water at Newburgh, NY. Information on the degree of hardness of the drinking water at Kunszentmárton is not readily available.

It is of interest to compare F, with its capacity to be genotoxic and to reduce melatonin secretion in animals and possibly humans, with electromagnetic radiation (EMR).

The late Dr Neil Cherry, Associate Professor in Environmental Health, Lincoln University, New Zealand, who died on May 31, 2003 at 56 from motor neurone disease, found that many studies indicate that radiofrequency/microwave (RF/MW) radiation[13,25-42] and extremely low frequency (ELF) fields[13,39,43-54] cause increased DNA strand breakage and chromosome aberrations. This effect has been reported in cell lines, human blood, animals, and living human beings. Epidemiological studies have shown the expected increases in cancer, miscarriage, and reproductive adverse effects.[55-62] Three plausible biological mechanisms may be involved.[13] Increased free radical activity and genetic damage to DNA and chromosomes may occur (i) as a response to the exposure[25-62] and (ii) because of an induced reduction in the free radical scavenger melatonin, for which there is both animal[63,64] and human evidence.[65-78] EMR may also (iii) alter cellular calcium ion homeostasis, which is involved in cell regulation, cell survival and apoptosis, DNA synthesis, and melatonin regulation.[79]

A recent 2013 paper by Hillman, Stetzer, Graham, Goeke, Mathson, VanHorn, and Wilcox examined the EMR effects of ground currents near electrical power lines on milk production of dairy herds. They found that production was reduced by eight independent electrical variables including transient voltage and harmonic distortion.[80] In a literature review, with 125 references, they noted that an electromagnetic field (EMF) proliferates and exacerbates many diseases. These include neoplasia with leukaemia and cancer of the central nervous system, breast, ovary, prostate, and testis. Other brain conditions affected are Alzheimer's disease and stroke. There also occurred cardiac arrhythmias, including atrial fibrillation,

and allergies, including asthma. They stated that EMF interrupts communication between cells, enzyme action, ATP energy transfer, homeostasis, and neuroendocrine control of the autonomic nervous system. Other effects were interruption of immune defence, reproduction, and the neuroendocrine response of the adrenals, thyroid, gonads, and other glands. Hillman et al. referenced that electrical exposure disturbed melatonin secretion patterns in blood by the pineal gland. They noted that weak ELF magnetic fields were genotoxic and may promote DNA damage.

The 29-member BioInitiative Working Group 2012 noted in 2007 that there was little doubt that ELF caused childhood leukemia.[81] They referenced a study which found that leukemia risks for young boys doubled at only 1.4 mG and above.[82] Children with leukemia in recovery had a poorer survival rate if their ELF exposure at home, or where they were recovering, was between 1 mG and 2 mG in one study and over 3 mG in another study.[81] In the 2012 Supplement to Section 1 of the BioInitiative 2012 report, it is noted that, with 42 epidemiological studies published to date, power frequency electromagnetic fields (EMF) were among the most comprehensively studied environmental factors in leukemia. Except for ionising radiation no other environmental factor was as firmly established to increase the risk of childhood leukemia. In considering RF EMR, Sage reported there was a consistent pattern of increased risk of glioma and acoustic neuroma associated with the use of mobile phones and cordless phones.[81,83,84] She also noted that sperm studies are showing DNA damage, impaired sperm quality, motility, and viability from cell phones on standby mode and wireless laptop use at exposures of 0.00034 $\mu W/cm^2$ to 0.01 $\mu W/cm^2$.[81]

The World Health Organization International Agency on Cancer Research (IRAC) classified both ELF-EMF, in 2001, and RF-EMR, in 2011, as Group 2B Possible Human Carcinogens.[81]

Thus both EMR and F share the capacity to be genotoxic and reduce melatonin secretion. Reduced melatonin secretion is seen to play a central role in the neoplastic and other diseases associated with EMR exposure.[13] Lai and Singh found that both melatonin and the spin trap compound alpha-phenyl N-tertiary-butyl nitrone (PBN) could prevent microwave-induced DNA damage suggesting that free radicals were involved in producing the lesions.[39,40] Any reduction in melatonin secretion might therefore be relevant to the response to cancer and possibly also in other human diseases such as atherosclerosis and the aging process.[13,14,39,40]

Another similarity, between EMR, at power line frequencies, and F, is that for both the threshold for no-effects has been seen to be zero exposure.

Zero exposure threshold for no-effects for EMR at power line frequencies:

> Cherry noted that two studies indicated that the safe ELF exposure level is zero.[13] A report by Savitz et al. of electric utility workers showed a dose-response relationship in mortality from myocardial infarction and arrhythmic heart disease with thresholds near zero exposure.[86] A second

study by van Wijingaarden et al. on the incidence of suicide in electric utility workers also showed a dose-response relationship that had a threshold at zero exposure.[87] These two studies suggesting a zero exposure threshold for no-effects for EMR are on electric utility workers exposed to US power line frequency EMR of 60 Hz (hertz, cycles per sec.).

Other studies indicate that for health it is necessary to have exposure to naturally occurring EMR at approximately 10 Hz.[88] The earth's surface and the ionosphere form an electrodynamic resonating cavity that produces micropulsations in the magnetic field at extremely low frequencies, from about 25 Hz down to 1 every ten sec. Most of the micropulsation energy is concentrated at approximately 10 Hz. Weaver found that persons shielded from these natural fields developed irregularities in various circadian rhythms affecting body temperature, sleep-waking cycles, and urinary excretion of sodium, potassium, and calcium.[88,89] Introducing an infinitesimal electric field, 0.025 volts/cm, pulsing at 10 Hz dramatically restored normal patterns to most of the biological measurements.[88,89] The circadian rhythms are dependent on the level of melatonin secretion by the pineal which is sensitive to the daily cyclic pattern in the earth's magnetic field.[90] Applying a magnetic field of 0.5 G (gauss) or less, oriented so as to add to or subtract from the earth's normal field, will increase or decrease production of pineal melatonin and serotonin.[88]

Zero exposure threshold for no-effects for F:

Spittle found that there was no threshold for F neurotoxicity in drinking water, and that the only assuredly safe level is zero.[91] Similarly, after reviewing the 2006 United States National Research Council report, *Fluoride in drinking water: a scientific review of EPA's standards*, Carton found that the amount of fluoride necessary to cause the adverse health effects of moderate dental fluorosis, stage I skeletal fluorosis, decreased thyroid function, and detrimental effects on the brain, in susceptible members of the population was at or below the dose received from the current levels of fluoride recommended for water fluoridation.[92] He recommended that the Maximum Contaminant Level Goal (MCLG) for fluoride in drinking water should be zero.

In addition, safety standards, based on studies showing evidence of harm, have been made for both EMR and F.

Evidence-based safety standards for EMR:

Cherry recommended in 2000 an outdoor public exposure limit at the boundary of properties for RF/MW radiation of 0.1 μW/cm^2 (microwatts per square centimetre).[13] This level, which reduces the health risk from outside to less than that associated with using a computer or being in a kitchen with a microwave oven on, has been adopted in Salzburg, Austria,[13,80] and Plenum Leganés, Spain.[93] It was endorsed by the BioInitiative Working Group in 2007 in their 1479-page report. They noted, "A precautionary limit

of 0.1μW/cm^2 (which is also 0.614 volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers, and PDAs [personal digital or data assistants] and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of Wi-Fi technologies, we also recommend that wired alternatives to Wi-Fi be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future." (Wi-Fi is a popular technology that allows an electronic device, such as a personal computer, video-game console, smartphone, digital camera, tablet, or digital audio player, to connect to the internet wirelessly using radio waves, via a wireless network access point or hotspot.)

For ELF EMR, the Group's 2007 conclusion was: "While new ELF limits are being developed and implemented a reasonable approach would be a 1 mG [milligauss] (0.1 μT) [microtesla, 1 μT = 10 mG] planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 μT) limit for all other new construction. It is also recommended that a 1 mG (0.1 μT) limit be established for existing habitable space for children and/or women who are pregnant."[81]

In a 2012 Supplement to the BioInitiative 2012 report it was noted that on a precautionary public health basis, a reduction from the BioInitiative 2007 recommendation of 0.1 μW/cm^2 for cumulative outdoor radiofrequency radiation (RFR) down to something three orders of magnitude lower (in the low nW (nanowatt)/cm^2 range) was justified.[81] A scientific benchmark of 0.003 μW/cm^2 or 3 nW/cm^2 for the lowest observed effect level (LOEL) for RFR was based on mobile phone base station-level studies. Applying a ten-fold reduction to compensate for the lack of long-term exposure (to provide a safety buffer for chronic exposure, if needed) or for children as a sensitive subpopulation yielded a 0.0003–0.0006 μW/cm^2 or 0.3–0.6 nW/cm^2. It was noted that these levels might need to change in the future as new and better studies were completed which might lower or raises the current observed "effects levels."[81]

Despite the evidence-based Salzburg Resolution on Mobile Telecommunication Base Stations of 0.1 μW/cm^2 for RF EMR having been

made 13 years ago in 2000 at the International Conference on Cell Tower Siting Linking Science and Public Health at Salzburg, Austria,[81,94] there is still a wide variation in the current international limits, e.g., Salzburg, Austria: 0.1 μW/cm^2; Bulgaria, Hungary, Russia, Switzerland: 2–10 μW/cm^2; PR China: 7–10 μW/cm^2; Italy: 10 μW/cm^2; Australia: 200 μW/cm^2; Canada, Japan, Germany, New Zealand, USA: 200–1000 μW/cm^2, and United Kingdom: 1,000–10,000 μW/cm^2.[95] In 2007 the current public safety standards were 1,000–10,000 or more times higher than levels now commonly reported in mobile phone base stations to cause bioeffects.[81] Using the current figure for bioeffects from mobile phone base stations[81] of 0.003 μW/cm^2 and the international range of limits of 0.1–10,000 μW/cm^2 the current public safety standards are 3–3,000,000 times higher than the levels causing bioeffects. A million-fold difference in safety levels between countries currently exists.

Evidence-based safety standards for F:

Spittle derived a threshold value for the occurrence of neurotoxicity of 0.1 mg F/L from a pool of eight studies.[88] This matched the prescient recommendation by Babbitt and Doland in 1939 to the American Water Works Association that the maximum level of F in drinking water should be 0.1 mg F/L because at least a tenfold margin of safety should be maintained.[96] At present countries vary widely also in their approach to fluoride levels in drinking water. Water fluoridation is practised in 25 countries: Argentina (19%), Australia (80%), Brazil (41%), Brunei (95%), Canada (44%), Chile (70%), China in the Special Administrative Region of Hong Kong (100%), Fiji (36%), Guatemala (13%), Guyana (62%), the Irish Republic (73%), Israel (70% but due to stop by mid-2014 following a decision on July 29, 2013, at the Supreme Court sitting as the High Court of Justice), Libya (22%), Malaysia (75%), New Zealand (62%), Panama (15%), Papua New Guinea (6%), Peru (2%), the Republic of Korea (South Korea, 6%), Serbia (3%), Singapore (100%), Spain (11%), the United Kingdom (11%), the United States (64%) and Vietnam (4%).[97] In contrast, in India, fluoride has been seen to be a poison and that, although slow acting, it is able to cause a variety of health problems, particularly in the Indian context, when the diet is deficient in a number of nutrients and the body is unable to combat fluoride poisoning effects.[98] Susheela noted that promoting fluoridation of dental products in India should be considered as a crime.[98] She considered that the US Environmental Protection Agency (EPA) set the guideline for fluoride in drinking water in the USA at 4.0 mg/L for "the strangest reason" and that the WHO guideline of 1.5 mg F/L being the "desirable" upper limit in drinking water was unsuitable. She observed that the Republic of Senegal in West Africa had reduced the upper permissible limit of F in drinking water from 1.5 mg/L to 0.6 mg/L.

The wide variation between countries in standards for both EMR and F may be related to bureaucratic difficulties in keeping up date with new research findings,

lobbying by industry for whom lower standards may appear to offer a financial advantage, and a misplaced trust in official reports by decision making authorities.[99]

Many such official reports have been published on the efficacy and safety of fluoridation.[100-102] Similarly, Cherry found that some reports on EMR could not be trusted.[13] In 2000 he said he had no respect for the position on the health effects of EMR of the World Health Organization (WHO) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP).[13] He noted that they were being managed and chaired by Dr Michael Repacholi in the mid-1990s and that very strongly held views that there were only thermal effects came through consistently and pervasively. Cherry noted that Repacholi had close links with industry and considered that under Dr Repacholi's chairmanship the ICNIRP consistently misquoted and misrepresented the evidence. In Cherry's opinion, the ICNIRP rejected all the epidemiological evidence on the grounds that every single epidemiological study occurred with mean exposure levels orders of magnitude below their thermally-based standard. Cherry considered that they were highly selective by using only a small proportion of the available studies in order to construct and defend their own case. He stated that they dismissed large, reliable, and well-defined studies as being ill-defined and unreliable and that they stated that studies did not show significant increases in central nervous system cancers even when they did with significant dose-response relationships. During this time, under Dr Repacholi's leadership, both the WHO and the ICNIRP maintained the thermal view despite the large and ever-growing body of scientific research that firmly and conclusively challenged this.[13] In 2012, Sage stated that safety standards based on heating were irrelevant to protect against EMF (electromagnetic field)-levels of exposure and that there was an urgent need to revise EMF exposure standards.[81] Research showed that thresholds for effects were very low and that the safety standards needed to be reduced to limit the biological responses.[81]

Oxman, Lavis, and Fretheim noted that the guidelines for developing WHO guidelines did not seem to be closely followed when WHO developed recommendations for member states with, for example, systematic reviews and concise summaries of findings being rarely used.[103]

Rao and Thakur have demonstrated that genotoxicity can be caused by fluoride and can be ameliorated by the antioxidants melatonin and alma (*Emblica officinales*, Indian gooseberry). Similarly, melatonin has been demonstrated to prevent genotoxicity from EMR.[39,40] Rao and Thakur note that the antioxidant activities of alma may not be attributed to its ascorbic acid (vitamin C) content alone but be due also to the presence of polyphenols such as ellagic acid, gallic acid, and various tannins. The daily use of vegetables and fruits as a source of antioxidants, rather than taking drugs or tablets containing nutrients, has been recommended by Susheela.[98] She lists the antioxidants as vitamin C, vitamin E, carotene, glutathione, quercetin, allicin, capasaicin, ellagic acid, gallic acid, epicatechin, lycopene, glucosinolates, lutein and zeaxanthin. She places alma as a

source of vitamin C and records that it can be consumed as a vegetable, pickle, chutney, drink, and fruit juice supplement.

The ability of both F and EMR to cause oxidative stress, generate free radicals, and produce genotoxicity has resulted in them have similar profiles of toxicity. For example, the sperm studies showing that EMR can cause DNA damage, impaired sperm quality, motility, and viability[81] parallel the studies finding toxicity from fluoride.[98,104,105] These overlapping effects suggest that not only might sources of EMR radiation need to be considered as a confounding factor when designing experiments to assess F toxicity but possible areas to investigate for F toxicity might be found by considering the findings from the extensive EMR research that has been documented. Some collaboration between the two fields of research has already occurred with the Comet assay developed by Singh and McCoy[36,37,39,40,106,107] for the detection of DNA damage from EMR being used to detect DNA damage from F.[108-110]

In 2000, it was noted by Cherry that eight animal studies and 15 human studies, involving both ELF and RF EMR, had been done on the effects of EMR on melatonin secretion.[13] In 2012, Sage wrote that 11 of the 13 published epidemiologic residential and occupational studies are considered to provide evidence that high ELF MF (magnetic field) exposure can result in decreased melatonin production.[81] The two negative studies had important deficiencies that may have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. In addition, new research indicates that ELF MF exposure, *in vitro*, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor.[81]

Relatively little work (one animal and two human) has been done on the effects of F on melatonin secretion. Newborns produce minimal melatonin, melatonin peaks in early childhood, puberty occurs as melatonin declines, melatonin continues to decline in middle age, and older people produce negligible amounts of melatonin.[14] Cancer is a chronic disease problem from accumulated genetic cell damage.[13] Latencies for children and soft tissue cancers are as short as a few years while for most cancers development takes 10–40 yr to develop.[13] Cancer rates rise rapidly with age over 65 yr because of the life-time of accumulated cell damage and the drastic reduction in melatonin that occurs after puberty.[13] Luke found that the inhibitory effects of F on pineal melatonin synthesis in the male gerbil ceased sometime after 11½ weeks and allowed the enzymic activity in the gerbil pineal to increase to normal values by 16 weeks of age.[15] It is unknown at present whether the build up of F in the human pineal to high levels, up to 21,800 mg/kg in calcified pineal tissue ash, interferes with pinealocyte function resulting in lower melatonin levels and possibly less ability to repair genotoxic damage from a variety of aetiological factors leading to raised cancer rates, including for breast cancer, and higher rates of Alzheimer's disease.[81] There is considerable *in vitro* and animal evidence that melatonin protects against Alzheimer's disease.[81]

In view of the effects that reduced melatonin may have, on protection from Alzheimer's disease, atherosclerosis, and cancer, as well as the uncertainty about whether increased fluoride ingestion is lowering the age of the menarche, the area warrants further research. Rao and Thakur's study in this issue of *Fluoride* is important for both its therapeutic implications and for stimulating further thought on the wider issues of F-induced genotoxicity and melatonin.

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