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# EXCITOTOXICITY: A POSSIBLE CENTRAL MECHANISM IN FLUORIDE NEUROTOXICITY

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**SUMMARY: Recent evidence indicates that fluoride produces neuronal destruction and synaptic injury by a mechanism that involves free radical production and lipid peroxidation. For a number of pathological disorders of the central nervous system (CNS), excitotoxicity plays a critical role. Various studies have shown that many of the neurotoxic metals, such as mercury, lead, aluminum, and iron also injure neural elements in the CNS by an excitotoxic mechanism. Free radical generation and lipid peroxidation, especially in the face of hypomagnesemia and low neuronal energy production, also magnify excitotoxic sensitivity of neurons and their elements. This paper reviews briefly some of the studies that point to a common mechanism for the CNS neurotoxic effects of fluoride and calls for research directed toward further elucidation of this mechanism.**

Keywords: Aspartate; Excitotoxicity; Fluoride neurotoxicity; Fluoroaluminum complexes; Glutamate; 4-Hydroxynonenal; Melatonin; Neurodegeneration; Peroxynitrite; Reactive nitrogen species; Reactive oxygen species.

## INTRODUCTION

Compelling evidence indicates that fluoride produces injury to the central nervous system (CNS) by several mechanisms. Of particular interest is the ability of fluoride to induce free radical generation and lipid peroxidation in the brain, especially in the hippocampus. In addition, fluoride enhances aluminum absorption from the gastrointestinal mucosa and across the blood-brain barrier. Of particular concern is the recent demonstration that fluoride readily forms a chemical complex with aluminum, similar to the phosphate ion, which is toxic to neurons at low concentrations and can act as an activator of G-proteins, a membrane link to second messenger activation.

While it appears that the toxicity of fluoride is secondary to many widely divergent and unrelated processes, there is compelling evidence that a central mechanism may be involved called excitotoxicity (Figure and Table).

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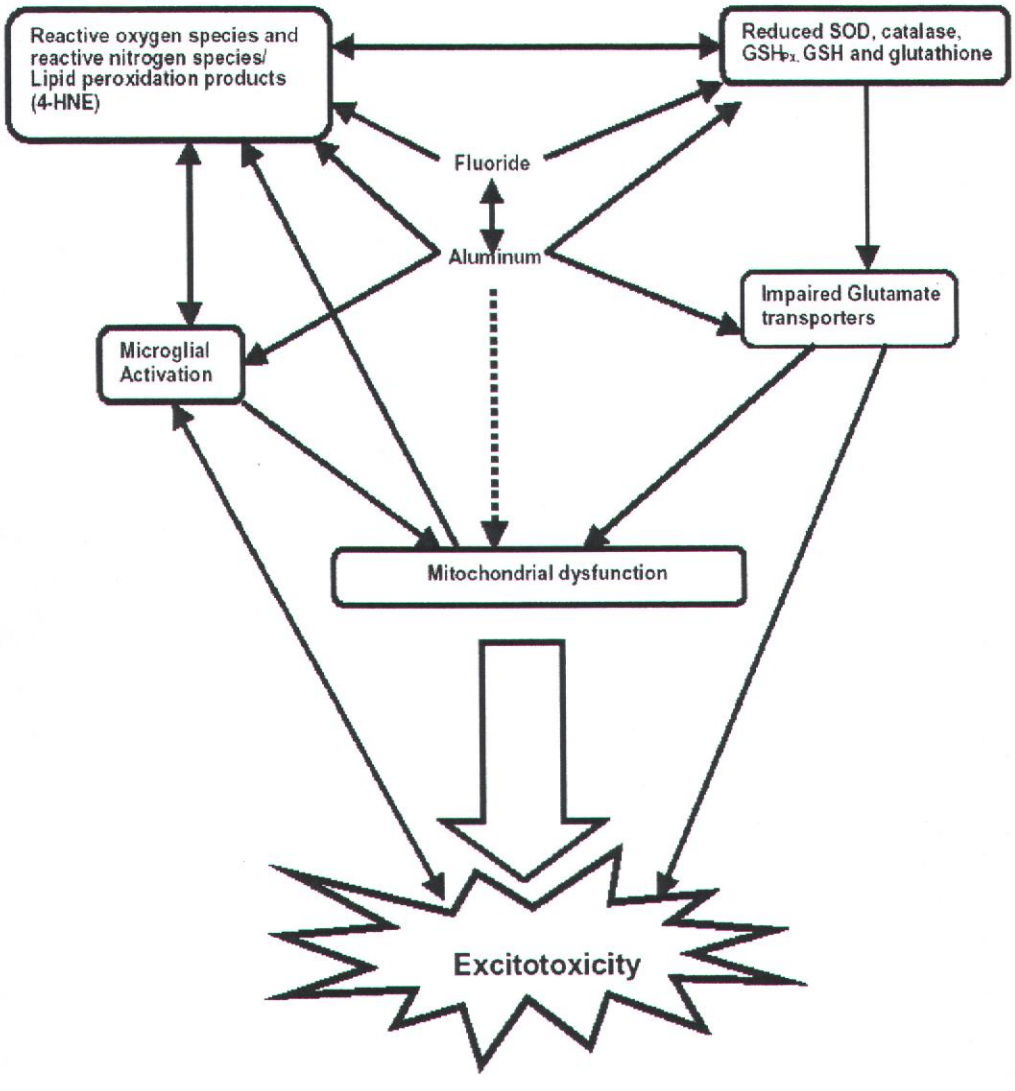


**Figure.** Possible mechanisms for neurodegenerative effects of fluoride and aluminum as related to excitotoxicity. The broken arrow represents the effects of both elements.

## WHAT IS EXCITOTOXICITY?

Excitotoxicity is a common mechanism seen in many neurological disorders, including strokes, brain trauma, CNS infections, autoimmune disorders, multiple sclerosis, heavy metal toxicity, brain tumors, and the majority of neurodegenerative diseases, such as Alzheimer's dementia, Parkinson's disease, and Lou Gehrig's disease (amyotrophic lateral sclerosis, ALS).[1] In a recent series of papers, I argue that excitotoxicity is also the central mechanism of autism and the Gulf War Syndrome.[2-4]

**Table.** Comparison of the effects of fluoride/aluminium and excitotoxicity

| | Fluoride/Aluminium | Excitotoxicity |
|---|---|---|
| Increased brain reactive oxygen species (ROS) and reactive nitrogen species (RNS) | yes | yes |
| Increased lipid peroxidation (LPO) | yes | yes |
| Decreased glutathione | yes | yes |
| Decreased superoxide dismutase (SOD) | yes | yes |
| Elevated brain ascorbate | yes | yes |
| Hippocampal apoptosis necrosis | yes | yes |
| G-protein activation | yes | yes |
| Synaptic injury | yes | yes |
| Impaired glutamate uptake | yes | yes |
| Microglial activation | ? for fluoride<br>yes for aluminium | yes |
| ROS in other tissues | ? for fluoride<br>yes for aluminium | yes |
| DNA injury | yes | yes |

The process involves accumulation of acidic amino acids in the synaptic cleft for a prolonged period. These special amino acids include cysteine, cysteine sulfinic acid, cysteic acid, and homocysteine, as well as the neurotransmitters glutamate and aspartate. The neurotransmitters glutamate and aspartate normally activate a series of glutamate receptors on the postsynaptic membrane that leads to neuronal excitation. In fact, glutamate is the most abundant neurotransmitter in the CNS and is responsible for attention, alertness, and learning. It is also the most neurotoxic.

If the excitatory amino acids are not removed quickly from the synaptic cleft, the postsynaptic neurons become overstimulated, leading to either synaptic destruction and dendritic retraction or, should the stimulation be prolonged and intense, neuronal destruction by both apoptosis and necrosis.[5] It is for these reasons that extracellular glutamate levels are carefully regulated by a series of glutamate transporters, which remove the glutamate for storage, either in the presynaptic neuron terminal or surrounding astrocytes (glia).[6]

This excitotoxic process was originally discovered by two ophthalmologists, Lucas and Newhouse in 1957[7] and given the name excitotoxicity by Dr John Olney in 1969.[8] Since its discovery, a great deal has been learned about the mechanism of excitotoxicity, the receptors involved, and the glutamate uptake system. In addition, much has been discovered about other toxins that can activate this destructive process. Recently, glutamate receptors have been found in numerous peripheral tissues, including the testes, lungs, pancreatic islet cells, cardiac nerves, ovaries, endothelial cells, immune cells, and bone osteoblasts.[9]

## COMMON MECHANISMS

### *1.Free radical generation*

Glutamate receptors are found in numerous types of neurons, including those that utilize other neurotransmitters, such as GABA (gamma-aminobutyric acid), dopamine, norepinephrine, and serotonin.[10] There are two basic types of glutamate receptors, ion-gated channels (ionotrophic) and metabotropic receptors.[11] Three ionotrophic receptor types have been identified, based on their affinity for selective agonists. These include N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate receptors. Neurons frequently contain more than one of these receptors types on the synaptic membranes.

The ionotrophic receptors control the passage of sodium, potassium, and calcium through membrane channels, which in turn initiates neuronal depolarization (excitation). Most important to the excitotoxic process is calcium accumulation within the cytosol following glutamate receptor activation. Intracellular calcium triggers numerous cellular reactions including the activation of nitric oxide synthase and protein kinase C.[12] These in turn can activate free radical generation and lipid peroxidation as well as eicosanoid activation, should glutamate persist too long in its receptor.[13] These processes play a major role in excitotoxic injury and neuronal death.

Three types of metabotropic receptors and eight subtypes of these receptors have been identified through cloning techniques. They operate mainly by GTP (guanine triphosphate) binding proteins or G-proteins. [14] When these receptors are stimulated by glutamate, the G-protein within the cell membrane is activated, which in turn activates several second messengers within the neuron, including IP3 (inositol 1,4,5-trisphosphate), cAMP (cyclic adenine monophosphate), or cGMP (cyclic guanine monophosphate). There is also evidence that they regulate intracellular calcium.[16] Two of the metabotropic receptors are thought to be neuroprotective and one is capable of triggering excitotoxicity.

Free radicals and lipid peroxidation products generated by excitotoxicity have been shown to damage dendrites and synaptic connections, and, if unrelieved, lead to neuronal destruction.[16] Likewise, free radicals caused by other processes have been shown to trigger excitotoxicity by impairing glutamate removal and by activating microglia, which contain abundant stores of glutamate.[17]

It has also been shown that one of the lipid peroxidation products, 4-hydroxynonenal (4-HNE), specifically impairs synaptic function and inhibits glutamate removal by the glutamate transport proteins.[18] This lipid peroxidation product, though less abundant than malondialdehyde, is significantly more neurotoxic. Any process that precipitates lipid peroxidation also precipitates the production of 4-HNE. Therefore, even if fluoride does not directly trigger excitotoxicity, it will do so indirectly by impairing glutamate removal and by generating reactive oxygen intermediates and lipid peroxidation products.

A study from China found that sodium fluoride significantly increased nitric oxide synthase (NOS) activity.[19] Interestingly, excitotoxins also stimulated NOS activity, which increases intracellular nitric oxide (NO) content. This is of particular importance because NO combines readily with superoxide forming the very powerfully toxic peroxynitrite radical, which plays a major role in all neurodegenerative diseases, primarily by damaging mitochondrial energy production, inhibiting glutamate re-uptake, and stimulating lipid peroxidation.[20-21] Fluoride has also been shown to inhibit superoxide dismutase, which would increase intracellular levels of the superoxide radical, the substrate for peroxynitrite formation.[22]

Another related neurotoxin, aluminum, is known to produce a dramatic increase in brain free radical generation and lipid peroxidation both directly and by increasing neuronal and glial iron levels.[23] In addition, melanin has a high affinity for aluminum, making neuromelanin-containing neurons in the *substantia nigra pars compacta* significantly more vulnerable to free radical and lipid peroxidation injury.[24] Aluminum accumulation and focal increases in reactive oxygen species and lipid peroxidation in this nucleus have been demonstrated in Parkinson's disease.[25]

Another mechanism by which fluoride might increase brain free radical generation and lipid peroxidation would be through activation of protein kinase C by a fluoroaluminum complex. It is known that a major mechanism by which glutamate induces excitotoxicity is activation of protein kinase C. Blocking this enzyme affords significant protection against excitotoxicity. Lead dramatically increases protein kinase C activity in a manner similar to glutamate, thereby triggering excitotoxicity.[26] Fluoride, in the form of silicofluorides in drinking water has been found to increase blood lead levels significantly, indicating an indirect connection between fluoride, free radical generation, and excitotoxicity.[27]

Because of the intimate connection between excitotoxicity, free radical generation, and lipid peroxidation, one can safely assume that fluoride can at least initiate the process indirectly and because of chronic exposure seen with water fluoridation, one would expect an eventual increase in neurodegeneration-associate disorders such as Alzheimer's dementia, ALS, and Parkinson's disease.

*2. Inhibition of antioxidant enzymes*

Closely connected with excitotoxicity-precipitated free radical generation and lipid peroxidation is the eventual depletion of antioxidant defenses. Several stud-

ies have demonstrated that fluoride toxicity, as well as excitotoxic injury, is associated with selective antioxidant depletion.[28-30]

Fluoride has been shown to inhibit certain antioxidant enzymes and molecules, such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, catalase, and glutathione.[31] This would not only increase free radical injury but would also enhance excitotoxicity, since reactive oxygen species as well as nitrogen species and lipid peroxidation products can trigger the excitotoxic process.[32] Antioxidant enzyme inhibition would necessarily enhance the toxicity of other neurotoxic elements, pesticides, herbicides, and environmental pollutants.

Another mechanism for magnifying the harmful effects of both fluoride and excitotoxins on the brain would be inhibition of melatonin. Melatonin, a hormone produced by the pineal gland, has been shown to have powerful neutralizing effects on free radicals and lipid peroxidation and to increase the levels of several of the antioxidant enzymes in the brain including SOD, glutathione reductase, glutathione peroxidase, catalase, and glutathione itself.[33]

A recent study has shown that fluoride significantly inhibits the release of melatonin from the pineal gland and that fluoride accumulates in the gland in very large concentrations in individuals drinking fluoridated water.[34] Ironically, glutamate and aspartate also powerfully inhibit melatonin release from the pineal gland and do so by a metabotropic receptor.[35] Conceivably, fluoride inhibits release of pineal melatonin by elevating glutamate levels. Since no research has been reported looking for this connection we do not know.

A recent study revealed that babies with the lowest melatonin production had the most neurobehavioral problems.[36] Melatonin levels are also lower in the cerebrospinal fluid (CSF) of Alzheimer's patients as compared with normal individuals.[37] The fact that fluoride lowers melatonin production would indicate that risk of neurodegeneration in both instances would be elevated.[38]

*3. Inhibition of mitochondrial energy enzymes*

Another connection between glutamate excitotoxicity and fluoride toxicity is related to inhibition of brain energy production. Several studies have shown that anything which suppresses neuronal energy production, especially mitochondrial energy production, greatly enhances excitotoxic sensitivity.[39-41] In fact, when neuronal energy production is low, even physiological levels of excitotoxins such as glutamate can trigger excitotoxicity.

Fluoride is also known to inhibit cellular energy producing enzymes, including mitochondrial electron transport enzymes. It does this both directly, as in the case of glycolytic and Kreb's cycle enzymes,[42] and indirectly, as in the case of the mitochondrial enzymes by the effect of peroxynitrite.[43] Vani and Reddy demonstrated suppression of both antioxidant enzymes and energy generating enzymes in female mice treated with 20 mg of fluoride/kg bw for 14 days.[22]

The importance of neuronal energy suppression by fluoride lies in the fact that that mitochondrial energy suppression is intimately connected as an early event to neurodegenerative diseases such as Alzheimer's dementia and Parkinson's dis-

ease.[44-46] Since fluoride can inhibit these enzymes, even in low concentrations, there is an increased likelihood that excitotoxicity plays a significant role in this process. Likewise, it should be appreciated that Mullenix *et al* have shown that fluoride accumulates in various brain areas of the rat, particularly the hippocampus, resulting in higher fluoride levels in the brain than are seen in the blood.[47] The hippocampus is one of the most sensitive areas of the brain to a multitude of neurotoxic events.

*4. Inhibition of glutamate transporters*

One of the most important ways glutamate concentrations are controlled in the nervous system is by a series of glutamate transport proteins. Thus far, five such transporters have been demonstrated by cloning techniques.[48] Of particular importance are GLAST (cloned glutamate/aspartate transporter) and GLT-1 (glutamate transporter-1). These transporters are associated with either the glial cells or the neurons themselves. The glial transporters (GLAST and GLT-1) bind to synaptically released glutamate and transport it to the interior of the glial cells. The neuronal transporters bind the glutamate and transfer it to the interior of the presynaptic terminal.

Considerable evidence points to impairment of these transporters as major players in neurodevelopmental disorders and neurodegenerative diseases.[49] The function of these transporters is altered by a number of commonly encountered toxins including mercury,[50] aluminum,[51] iron,[52] cytokines,[53] eicosanoids (PGE2),[54] and 4-HNE.[55] In fact, mercury has been shown to inhibit the glutamate transporters at concentrations below those that are cytotoxic.[56] Anything that increases free radical generation and lipid peroxidation impairs glutamate transport.

Aluminum inhibition of glutamate transporters is of special interest because of the frequent and ready interaction of aluminum and fluoride to form a biologically reactive complex. Although no one has apparently examined the occurrence of fluoride-aluminum complexes as the common inhibitor involved, the possibility is quite high. This is because of the chemical avidity of fluoride for aluminum and the fact they frequently occur together in nature.

Even without the direct involvement of a fluoroaluminum complex, the fact that fluoride is known to cause a seven-fold increase in the absorption of aluminum past gut barriers is of significant concern.[57] In addition, fluoride enhances the passage across the blood-brain barrier. In several studies, fluoride added to drinking water doubled brain aluminum levels, thus increasing the likelihood of glutamate transporter inhibition.[58,59]

Aluminum glutamate, which is formed in the GI tract, has been shown to alter the blood-brain barrier making it more permeable to normally excluded toxins.[60] In addition, it enhanced both aluminum and glutamate concentrations in the brain, significantly increasing the risk of excitotoxicity.

## THE ALUMINUM-FLUORIDE CONNECTION

As mentioned in the introduction, aluminum interacts with fluoride to form a fluoroaluminum complex that mimics phosphate groups in biological systems.[61] By this mechanism, it could also activate the G-proteins in cell membranes. As we have seen, the metabotropic receptors are activated by a G-protein mechanism. In addition, numerous cells in the body utilize the G-protein second messenger receptor system, including endothelial cells, lymphocytes, osteoblasts, other neurotransmitters (dopamine, norepinephrine, acetylcholine, serotonin, neuropeptides, and opioids), and glucagon.

Activation of metabotropic excitatory receptors by an aluminum-fluoride complex could initiate excitotoxicity as shown by Lan and coworkers.[62] Because the aluminum-fluoride complex accumulates in the brain, it would also be expected to cause prolonged neurotoxicity, leading eventually to neurodegeneration and synaptic loss.

The aluminum-fluoride complex has been shown to produce neuronal loss in the CA1 and CA-4 areas of the hippocampus when given to animals as 0.5 ppm in drinking water.[59] The toxic effect may be related to a combination of effects, including impairment of energy-producing enzymes, impaired dephosphorylation of hyperphosphorylated tau-protein, increased neuronal iron concentration, elevated free radical and lipid peroxidation levels, and impaired DNA repair, all of which are related to excitotoxicity.

Another toxic effect of aluminum, and possibly a fluoroaluminum complex, is the activation of microglia. These are resident immune cells within the nervous system, which are normally quiescent, but are easily activated by a number of environmental and biological agents, such as viruses, mycoplasma, bacteria, aluminum, mercury, and several pesticides.[63]

Once activated, microglia generate and secrete a number of neurotoxic compounds, including two powerful excitotoxins: glutamate and quinolinic acid.[64] The combination of excitotoxin secretion and cytokine production greatly increases the concentration of free radicals and lipid peroxidation products in the brain. No one has looked at the possibility of fluoride-induced microglial activation. Yet, one would expect the fluoroaluminum complex to activate microglia, since aluminum alone is a powerful activator.[65]

Chronic microglial activation has been associated with a number of neurodegenerative processes, including strokes, multiple sclerosis, brain trauma, experimental allergic encephalomyelitis (EAE), Alzheimer's dementia, Parkinson's disease, and ALS.[3] Because both aluminum and fluoride accumulate in the brain and have their highest concentrations in the hippocampus and neocortex, one would expect chronic microglial activation as well. At least one study noted reactive gliosis (microglial activation) in association with fluoride brain toxicity.[66]

## FLUORIDE: A SPECIAL DANGER TO THE DEVELOPING BRAIN

The brain undergoes one of the fastest growth and development rates of any portion of the human body during embryogenesis. This occurs especially during the last trimester and first two years of life, a period called the brain growth spurt. This involves not only the rapid development of synaptic connections (synaptogenesis) and pathway development, but also refinement of all of the synaptic connection made during this period. One way glutamate does this is by stimulating the growth cones that guide neural pathways to their intended destination. The brain develops far greater synaptic connections than are needed during this "brain growth spurt" and as a result, synaptic connections are removed in a process referred to as pruning.

Connected to this pruning process, as well as to synaptogenesis and pathway development, is the level of glutamate within the brain. The rise and fall of brain glutamate levels during development controls these processes, and is finely tuned throughout brain development.[67] Too much glutamate overprunes the synapses and dendrites, whereas too little results in an excess of un-needed connections.[68] Both can result in severe neurodevelopmental problems.

Recent studies have revealed that the glutamate transport proteins also play a significant role in the development of the brain.[69,70] As shown by these studies, anything that alters transporter function can affect brain development. By interfering with neuronal energy production, neurotransmitter levels (especially glutamate), free radical generation and growth cone function, fluoride can have significant harmful effects on neurodevelopment.

In addition, fluoride has also been found to inhibit thyroid function and thereby alter early neuron migration in the developing fetus.[71] This can result in irreversible changes in the fetal brain.

## A CALL FOR FURTHER RESEARCH

It is obvious from this short review that more research needs to be done in this area. We need data on both the effects of fluoride and fluoroaluminum on the glutamate transporter proteins and on the exact mechanism of free radical generation being caused by fluoride. In addition, we need studies to see if fluoride can cause chronic microglial activation and neurodegeneration.

Because of the growing number of studies showing a strong connection between aluminum accumulation in the brain and neurodegenerative diseases, studies need to be done to see if the aluminum in neurofibrillary tangles and senile plaques is in fact fluoroaluminum. Further studies are also needed to see if fluoroaluminum passes along olfactory axons into the entorhinal area as has been demonstrated for aluminum itself.[72] This would not only provide direct access to the area of the brain showing the earliest changes of Alzheimer's dementia, but would allow lower concentrations in the drinking water to produce higher concentrations in the hippocampal area than would be attainable from blood.

In addition, special studies are needed using silicofluorides to see if their toxicity to the nervous system differs from that of sodium fluoride. Along this same line, we need data on the possibility of additive and even synergic toxicities when fluoride is combined with mercury, lead, cadmium, and other known neurotoxins.

Although progress has been made on nutrient-based neuroprotection against fluoride toxicity, more research needs to be pursued.[73-77] Chinoy and Sharma found that both vitamin E and $D_3$ reversed the toxic effect of fluoride on male reproductive organs and that a combination of the two antioxidants completely reversed the toxicity.[78] In a recent study, Chinoy and Shah found that a combination of vitamin C and E and calcium could reverse the toxic effects of both fluoride and arsenic on multiple biochemical parameters, including suppression of dehydroascorbic acid, glutathione, glutathione peroxidase, and SOD in the brains of mice.[79] If excitotoxicity indeed plays a significant role in fluoride toxicity, we need to apply some of the methods used to protect against excitotoxicity, such as increasing the intake of methylcobalamin, melatonin, selenium, the B vitamins, vitamins C, E, D, and K, along with metabolic stimulants such as pyruvate, malate, CoQ10, acetyl-L-carnitine, R-lipoic acid, and ginkgo biloba. Of special importance is supplementation with magnesium, which has been shown to block the NMDA glutamate receptor and decrease free radical production.

One area of particular interest is the use of flavonoids as neuroprotectants. Plant flavonoids are known to be the most versatile and powerful antioxidants known, and one of the few antioxidants that will neutralize peroxynitrite.[80] In addition, they can chelate metals, reduce inflammation, block eicosanoid production, and inhibit enzymes such as protein kinase C, which is critical to excitotoxicity and lead neurotoxicity.[81] A recent study by Juzyszyn and co-workers found that quercetin sulfonate, a water-soluble form of the flavonoid quercetin, protected liver and kidney cells from ammonium fluoride suppression of mitochondrial energy production.[82]

Finally, we need more data on the concentration and accumulation of fluoride in other calcified areas of the brain beside the pineal gland. For example, calcification of the basal ganglion is seen in a small number of individuals. In the past, this was considered an asymptomatic condition occurring in 0.3% of the population examined.[83] While basal ganglion calcification has been noted in a number of disorders, of particular interest is its appearance in Down's syndrome. One study on autopsied Down's brains found calcification in 45% in the area of the basal ganglion and increased calcification there with increasing age.[84] Newer studies have shown that a significant number of these individuals have symptoms related to basal ganglion dysfunction as well as neuropsychiatric disturbances.[85] In addition, recent studies has shown that excitotoxicity induces calcification deposits in the brain, which also contain aluminosilicates.[86] Should these calcifications accumulate fluoride in high concentrations as found in pineal calcifications, one would expect damage to adjacent neurons and glia. With widespread fluoridation of drinking water, one would also expect higher fluoride concentrations in these calcified structures than in the past.

It is obvious from this review that there is an intimate connection between the neurotoxicity of fluoride, aluminum, and glutamate that needs further attention. It is also obvious that excitotoxicity plays some role in this process, perhaps a central one.

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