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Neurotoxins and Autism

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Abstract

Recently, a great concern has risen about the increasing prevalence of autism as a neuro-developmental disorder. Environmental factors as significant contributors to children's health through a wide range of routes are linked to remarkable increases in this disorder. It is well known and accepted that young children are more vulnerable to environmental toxins, compared to adults. Modern day lifestyles with more mercury and lead exposures, fast food, cell phones, and microwaves place children at higher risk of neurotoxicity. Moreover, a huge number of synthetic chemicals termed as high-production-volume (HPV) chemicals are found in many products such as medications, cosmetics, building materials, plastic, and car fuels. These HPVs highly contribute to brain damage in developing infants. Other environmental toxins include thalidomide, valproic acid, misoprostol, and many infectious agents among which are pathogenic bacteria or their metabolites are found to be neurotoxic and/or linked to incidences of autism. This chapter summarizes the most important routes of exposure to environmental neurotoxins and explains how these toxins are related to the remarkable increase in the prevalence of autism through different etiological mechanisms such as oxidative stress, neuroinflammation, impaired neurochemistry and glutamate excitotoxicity.

Keywords: neurotoxins, heavy metals, mercury, *Clostridium difficile* aesthetic drugs, valproic acid, insecticides, herbicides, cell phones

1. Introduction

Recently, a great concern has risen about the increasing prevalence of “autism as a neuro-developmental disorder” characterized by impaired social interaction, communication, and

repetitive behavior. Environmental factors as significant contributors to children's health through a wide range of routes are greatly involved in the remarkable increase of autism spectrum disorder (ASD). It is well known and accepted that young children are more vulnerable to environmental toxins compared to adults because they breathe more air and consume more food relative to their body size in order to meet requirements of growth and development. Common hand-to-mouth behavior among infants and even young children of course increases their risk of exposures to environmental toxins.

In relation to the modern lifestyles—cell phones, microwaves, mercury and lead exposures, plastic, fast food—place children at higher risk of neurotoxicity that might lead to brain damage during early development. Moreover, environmental toxins such as misoprostol, valproic acid (VPA), and thalidomide have been reported as neurotoxins and linked to ASD. Pathogenic bacteria or their metabolites, cytomegalovirus, rubella, toxoplasmosis, and herpes simplex have also been characterized as neurotoxins which greatly contribute to autism.

This chapter summarizes the most important routes of exposure to environmental neurotoxins and explains how these toxins are related to the remarkable increase in the prevalence of autism. Moreover, the role of the stages of development and timing of exposures (prenatal, perinatal, and postnatal) will be discussed.

Based on the recorded biomarkers of autism, the role of selected environmental toxins in the induction of oxidative stress, neuroinflammation, impaired neurochemistry, and glutamate excitotoxicity as etiological mechanisms related to autism will be highlighted and illustrated.

1.1. Heavy metals as neurotoxins

A relationship between rises in environmental levels of Hg and the increase in both rates of autism and special education students has been reported [1]. In an attempt to find the relationship between elevated mercury levels and oxidative stress as etiological mechanisms in autistic individuals, Sajdel-Sulkowska et al. [2] found that mercury concentrations in the cerebellar areas of the brain were positively correlated with neurotrophin-3 (NT-3), as an oxidative stress marker. Khan et al. reported on NT-3 associated with much higher levels in autistic patients but without an association with Hg levels in blood, which was nonsignificantly different between autistic and control subjects [3]. This suggests that the same concentration of Hg may promote oxidative stress only in autistic patients but not in control subjects. In relation to Hg levels in hair as an indicator of neurotoxicity in autistic patients, the "poor excretor theory" asserts that autistic children are more prone to accumulate Hg because they are unable to readily excrete it when compared to age- and gender-matched controls [4]. However, other studies recorded that the higher the Hg levels in hair, the worse the autism symptoms [5]. A direct relationship between elevated blood levels of Hg and the degree of autism severity according to scales childhood autism rating scales (CARS), social responsiveness scales (SRS), and short sensory profile (SSP) was ascertained [6, 7].

In an attempt to better understand the role of heavy metal neurotoxicity, investigations of air pollution, Hg, lead (Pb), and arsenic (As) have been shown to stimulate oxidative stress and

inflammation in humans, which may contribute to the pathogenesis of autism [8, 9]. Based on multiple studies, Pb, Hg, and As were recorded as neurotoxins related to autism [10–12]. Associations between autism prevalence and proximity to industrial facilities were ascertained by Dickerson et al. [13]. Most recently, a disruption of complex neuro-immune signaling as a mechanism necessary for neuronal migration and brain growth was accepted to be a possible mechanism to cause Hg-induced brain damage [14].

Gut microbiota, which is known to be remarkably modulated in autistic patients, can be easily related to the elevated level of Hg. Gut microbes can modulate Hg via either methylation of less toxic inorganic Hg, Hg^{+2} , or demethylation (i.e., detoxification) of methylmercury (MeHg) [15–18]. In studying bacterial diversity in relation to the MeHg level, Rothenberg et al. found that, among the studied bacterial species *Clostridiales*, *Subdoligranulum*, and *Akkermansia* spp., positive correlations for stool MeHg, hair total mercury (THg), and stool inorganic Hg were evident, while negative correlations for *Streptococcus* were determined using Spearman's and/or Pearson's [19]. These relative effects were related to approximately tenfold higher *Clostridium difficile* levels in the stool from autistic subjects, which may help to support the use of probiotics to ameliorate MeHg elevations which may lead to the development of autistic features [20]. This is summarized in **Figure 1**.

In relation to the antioxidant and protective effect of selenium (Se), a recent review was written by Bjorklund and Causey on the molecular interactions between Hg and Se which result in neurotoxicity. Selected studies revealed associations between autism and Hg and Se concentration changes in hair and/or nails of autistic patients [21]. These studies reported significant increases in the levels of Hg and concurrent decreases in the levels of Se in hair and nails and

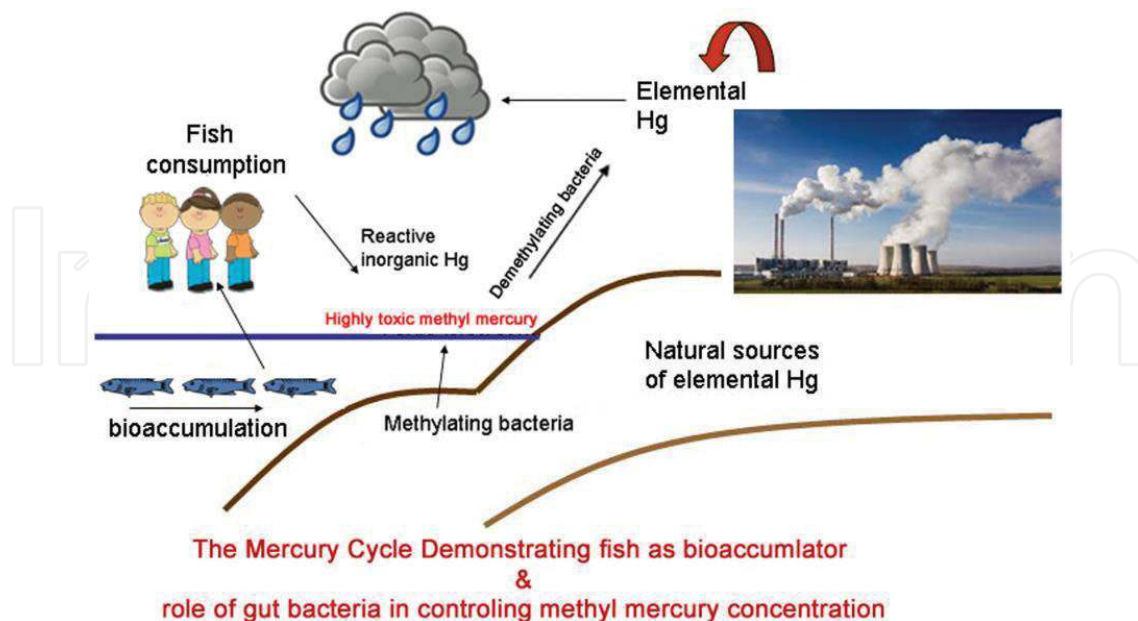


Figure 1. Role of fish in the bioaccumulation of mercury (Hg) and role of methylating and demethylating bacteria in the control of methyl mercury (MeHg) concentration as environmental neurotoxin related to autism.

the association of these changes with the severity of an autistic phenotype [5, 22]. Other studies showed significant elevations in Hg in hair [23] and urine [22] of autistic patients without significant decrease of Se concentration or Se/Hg ratio. Moreover, some studies reported a lower Zn/Cu ratio in blood from autistic subjects as compared to healthy control subjects [24, 25] yet strong causal relationships for Hg neurotoxicity.

1.2. Anesthetic drugs as neurotoxins in autism

An early study of the role of anesthesia in relation to neurotoxicity was described by Ikonomidou et al. [26]. They observed the effects of N-methyl-D-aspartate (NMDA) antagonist injections in rat pups, which led to acute postnatal neuronal apoptosis [26]. Also, they hypothesized that anesthetics such as ketamine blocked endogenous glutamate stimulation via NMDA receptors, leading to neuronal apoptosis. Apoptosis of neurons as an invasive marker of neurotoxicity was repeatedly demonstrated especially in animals that received multiple, high doses of ketamine during periods of developmental vulnerability [27, 28]. Upregulation of NMDA receptor expression levels in response to ketamine administration may modulate intracellular calcium homeostasis, possibly leading to apoptosis [29, 30]. Additionally, activation of gamma-aminobutyric acid (GABA) receptors through the inhalation of isoflurane-induced neurotoxicity in hippocampal culture cells was also associated with excessive neuronal influx of calcium [31]. Based on observation, it was suggested that anesthesia-induced neuronal toxicity may appear secondary to loss of calcium homeostasis within mitochondria as evident by mitochondrial dysfunction, accumulation of reactive oxygen species (ROS), and overexpression of caspases as pro-apoptotic markers (**Figure 2**) [32–34]. Although the actual mechanism for ROS accumulation was not identified, administration(s) of either an antioxidant or a

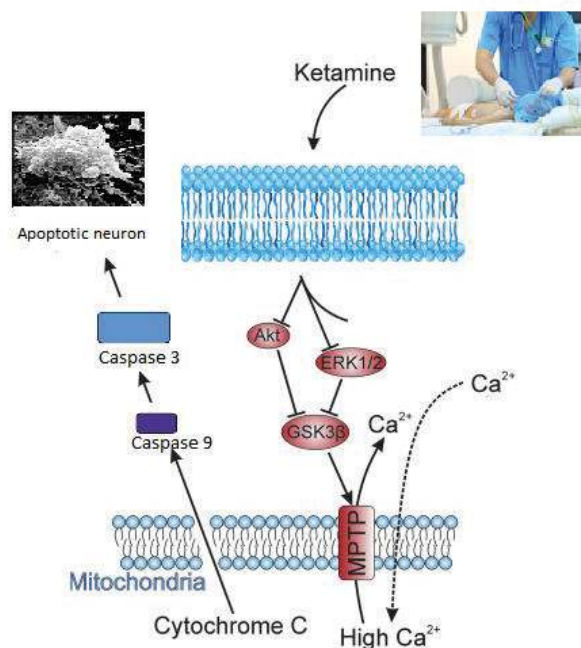


Figure 2. Neurotoxic effect of ketamine through loss of calcium homeostasis leading to neuronal death.

mitochondrial protectant prevented anesthesia-induced neuronal apoptosis and downstream cognitive impairment in developing rat brains [33, 35].

1.3. Antiepileptic drugs (AEDs) as neurotoxins in autism

The use of antiepileptic drugs (AEDs) by pregnant mothers was found to be involved in major congenital abnormalities seen in neurodevelopmental disorders among which is autism [36]. Animal studies demonstrated that exposure to AEDs may result in neurotoxicity; for example, VPA, phenytoin, and phenobarbital, cause impaired neurodevelopment after prenatal exposure [37]. Despite the therapeutic effects of VPA, it is also associated with neurotoxicity [38] as evidenced in in vitro models. VPA neurotoxicity is usually related to the increased of ROS production, as a critical contributor to brain damage and dysfunction [38–40]. Mitochondrial dysfunction has been proposed as one of the most common deleterious effects of VPA neurotoxicity [41]. In relation to autism, VPA is most commonly related to the etiopathology and development of most of phenotypic features of autism. Many cases, population database studies, prospective studies, and retrospective studies, ascertained increased incidences of ASD and cognitive deficits in VPA-exposed children with a reported risk of 6–8% [42–45].

Chaudhary and Parvez observed a significant decrease in acetylcholinesterase (AChE) activity [46]. The inhibition of AChE activity by VPA in the cerebellum and cerebral cortex results in the accumulation of ACh at cholinergic synapses, leading to ACh receptor stimulation, decreased cellular metabolism, induction of cell membrane alterations, and disturbances in neuronal activities [47]. VPA-inhibited Na^+/K^+ -ATPase is an enzyme controlling the active transport of CNS sodium and potassium ions in a dose-dependent manner [48]. The marked inhibition of Na^+/K^+ -ATPase activity may compromise neurotransmission, leading to partial membrane depolarization and excessive Ca^{2+} entry inside neurons which may in turn induce glutamate excitotoxicity. This can be ascertained through the recent work of Kim et al. in which agmatine was used to treat VPA-induced animal models of neurotoxicity, where to, the amelioration of glutamate excitability improves sociability and decreases the repetitive behavior appeared as two important autistic features [49]. In a recent study by Videman et al., carbamazepine, oxcarbazepine, and VPA were associated with impaired early language abilities at the age of 7 months. In contrast, face perception or social attention may be less affected by the neurotoxic effects of the studied AEDs [50]. Previously, the association between prenatal exposure to AEDs and the increased risk of cognitive impairment and ASD was detected at ages of 2 to 6 years.

1.4. Clostridium neurotoxins and autism

It is well accepted that healthy gut microbiota provides an effective barrier against colonization by opportunistic bacteria [51, 52]. This protective microbiota is severely disrupted with abuse of broad-spectrum antibiotics frequently administered during early childhood [53]. *C. difficile* produces two exotoxins: toxin A and toxin B. Acting together, these toxins damage intestinal mucosa and cells and result in watery diarrhea, which is the primary clinical symptom of *C. difficile* infection [54]. In addition to *C. difficile*, *Clostridium tetani* is another opportunistic pathogen that can lay dormant in spore form for long periods of time. Both species

produce cytotoxins known to cause cellular damage. Additionally, both *C. tetani* and *C. difficile* produce phenolic metabolites [55]. Some toxigenic strains of *C. tetani* produce an extremely potent neurotoxin. Clostridia toxins can enter the circulation and accumulate at nerve terminals through binding to host-independent receptors [56, 57]. Following endocytosis of the neurotoxin-receptor complex, acidification of the presynaptic vesicle triggers a conformational change in the N-terminal translocation domain. Acidification is mediated by the vesicular ATPase proton pump, whose function is to ensure the reuptake of neurotransmitters into the synaptic vesicle. This might provide a plausible mechanism for the abnormal reuptake of glutamate in autistic patients. Several studies have assessed the fecal flora of autistic and control individuals, reporting an overgrowth of pathogenic bacterial species in autistic patients when compared to controls. Among these species is *Clostridium* species, including *C. difficile* [58–60]. In addition, Parracho et al. found a higher incidence of the *Clostridium histolyticum* group in the fecal flora of 58 ASD children compared to 10 healthy children [61].

Clayton hypothesized that impaired gut microbiota can be linked to autism through the abnormal gut bacterial metabolism of phenylalanine and tyrosine resulting in the production of P-cresol, with *C. difficile* as one of the most notable p-cresol producers [62]. Overgrowth of *C. difficile* can be linked to the etiology of autism through the inhibitory effect of p-cresol on dopamine β -hydroxylase as a rate-limiting enzyme of dopamine metabolism [63]. Moreover, *C. difficile*-induced production of p-cresol can inhibit sulfonation as a detoxification mechanism of special importance when considering neonatal inactive glucuronidation as an alternative detoxification reaction for xenobiotic excretion [64, 65]. Based on this information, p-cresol may be linked to autism through impaired gut microbiota and the overgrowth of *C. difficile* [66]. **Figure 3** summarizes the role of *C. difficile* overgrowth in the etiology of autism. Hsiao et al. reported that bacterial toxin-induced metabolic changes can trigger autistic behavior. Maternal immune-activated (MIA) females produce offspring

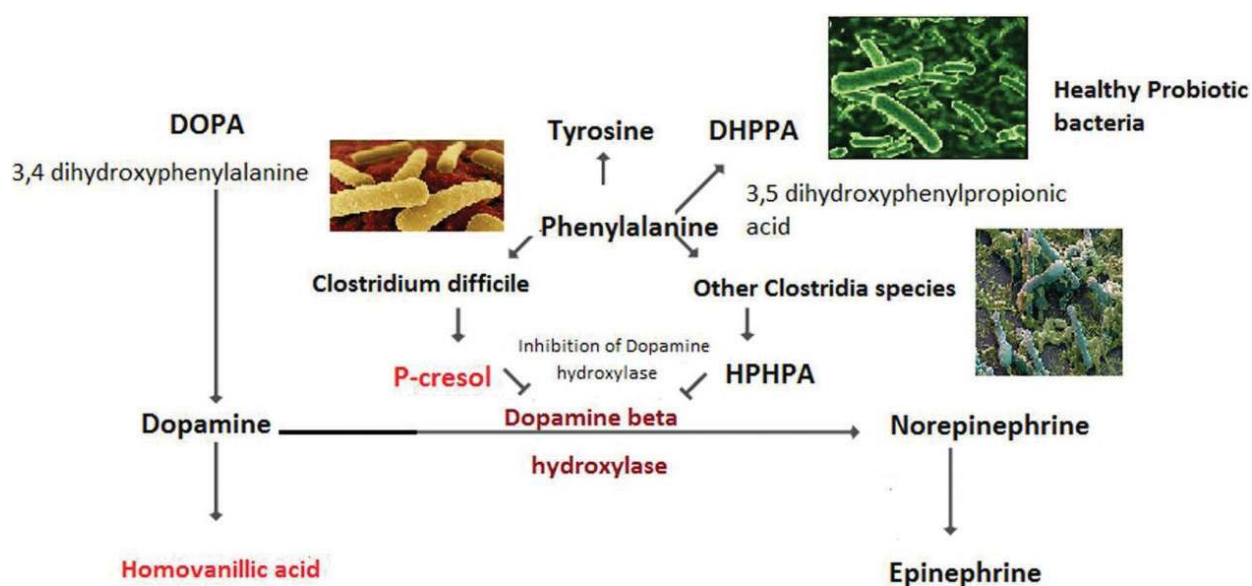


Figure 3. Role of *C. difficile* overgrowth in the etiology of autism through the inhibition of dopamine-beta hydroxylase by p-cresol as bacterial metabolite of tyrosine and phenylalanine.

with impaired communicative and social behavior representative as autistic features [67]. Moreover, MIA offspring displayed altered gut microbiota and leaky gut. Treatment of mice with *Bacteroides fragilis* was effective in restoring normal gut permeability and reduces anxiety-like behavioral deficits in this autism model [67]. Most recently, Yang and Chiu reported that through the soluble NSF attachment protein receptor (SNARE) complex, clostridial neurotoxins block neurotransmission to or from neurons. In addition, the gut microbiota produces molecules that act on enteric neurons to reduce gastrointestinal motility and metabolites that stimulate the “gut-brain axis” to alter neural circuits and brain function and behavior [68]. Aljarallah reported that water extract of myrrh plant demonstrates high antimicrobial effect against *C. difficile* strains, which could support its use as a natural product for the amelioration of *C. difficile* in autistic patients [69].

1.5. Pesticides as neurotoxins in autism

By using retrospective epidemiological studies, environmental factors such as pesticides have been linked to autism. Experimental research in mouse cortical neuron-enriched cultures exposed to hundreds of chemicals commonly found in the environment and on food showed how such chemicals greatly affect brain development. Pearson et al. have been found that rotenone, a pesticide associated with Parkinson’s disease risk, and certain fungicides, including pyraclostrobin, trifloxystrobin, famoxadone, and fenamidone, produce transcriptional changes in vitro that are similar to those seen in brain samples from humans with autism [70]. These chemicals stimulate ROS production and disrupt microtubules in neurons, effects that can be reduced by pretreating with a microtubule stabilizer and an antioxidant such as sulforaphane. In this study, 283 autistic children showed neuronal tube defects with potential relationships between maternal residential proximity and agricultural use of neurotoxic pesticides [71].

Organophosphates (OPs) are the most generally utilized pesticides in agriculture, as well as bug sprays in residential, commercial, and industrial settings. Fetus may be exposed to OPs via the placenta or infants through breast milk, food, and inhalation. These small children appear particularly vulnerable to OPs and oxidative stress compared to adults, because of their lower activity levels of the enzyme paraoxonase, involved in OP inactivation and lipid peroxide degradation [72]. This enzyme was found to be significantly lower in autistic patients compared to healthy controls [73]. Prenatal exposure to OPs has been connected to neurodevelopmental disorder, which appears to be maintained during childhood, including deficits in cognitive abilities, working memory, and perceptual reasoning [74–76]. Attention deficits, receptive language, social cognition problems, reward, and behavioral dysfunction have been correlated with lower intelligence quotient (IQ) scores in humans prenatally exposed to chlorpyrifos [76]. Prenatal OP exposure has also been linked to increase autism risk [77]. Acetylcholinesterase (AChE) has been shown to be inhibited with OPs, determining excessive cholinergic transmission; however, OP’s main neurotoxic actions are seemingly exerted by their axon metabolites [78].

There is a growing body of evidence that links the exposure to organochlorines (OCs) and autism. Despite their neurotoxic liabilities, OCs are frequently used in agriculture. The

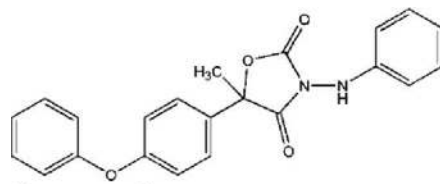
association between autism and pesticide exposure during the third trimester has been observed from mothers living near agricultural areas where pesticides were used [79]. Shelton et al. found that increased exposure to insect repellent can lead to autism [80]. Eskenazi et al. and Rauh et al. reported that autistic children had higher OP metabolites during early to mid-pregnancy [77, 81]. Case-control studies reported that exposure to imidacloprid, insecticide, through the consistent use of flea/tick pet treatment throughout pregnancy period was associated with ASD [79, 82]. Rauh et al. reported that children exposed to OP insecticides showed psychomotor and mental development delays, attention, hyperactivity disorders, and pervasive developmental issues by 3 years of age [81].

Robert and English (2012) described ASD and applications of OCs in proximity to maternal residence before, during, and after pregnancy. Bayesian model as a flexible step function was formulated to measure the time that is needed by pesticide to affect fetus or children for mother who lived near agricultural area [83]. The association between autism and OCs was high, and the time of this association was extending from approximately 4 months prior to fertilization to 8 months into pregnancy. Roberts et al. have suggested that the risk of ASD is increased by 6.1-fold in children with maternal exposure to OCPs during the first trimester of pregnancy, a key period of gestation and neurodevelopmental processes in neonates [84]. Here, 465 children were enrolled in retrospective study to assess pesticide type, exposure time, and residential distance from pesticide application. This study reported an association between prenatal exposure to dicofol and endosulfan pesticide during the 8 weeks immediately following the time of cranial neural tube, and increased risk of autism in children of mothers who lived within fields that had the highest quartile of estimated pesticide exposure compared with children whose mothers lived more than far from exposure, and therefore had the lowest exposure levels [84].

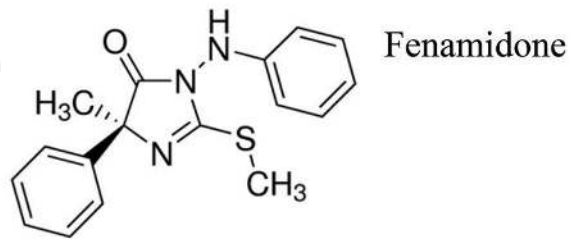
Chlorinated biphenyl (CB) is used as dispersant in pesticide [85]. Because is a CB are pollutant with potentially persistent immunological and neurological effects [86, 87]. It has also been reported that CB can increase the production of ROS and cytotoxicity [88]. The neurological and immunological abnormalities as well as oxidative stress due to CB exposure have also been observed in autistic children [89–91]. **Figure 4** presents structures for selected insecticides linked to neurotoxicity and autism.

1.6. Endocrine-disrupting chemicals

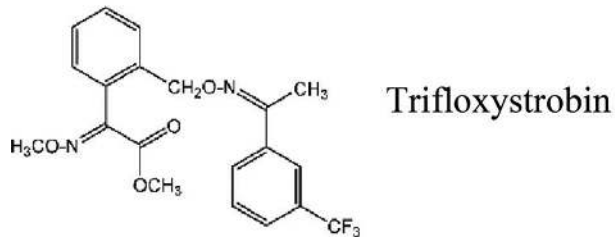
Endocrine-disrupting chemicals (EDCs) such as polychlorinated biphenyl (PCB), polybrominated diphenyl ethers, bisphenol A (BPA), dioxins, and phthalate have strong associations with neurological disorders [92]. These EDCs are able to interfere with hormone functions because they may alter hormone-dependent processes and/or disrupt endocrine gland function. Certain EDCs are able to alter synaptic function and neural networks [93]. The EDCs are also termed neural-disrupting chemicals since they may increase the prevalence of neurodevelopmental disorders including autism. Prospective epidemiological studies are warranted to better understand EDC-related effects in humans [94]. Braun et al. found that midpregnancy BPA concentrations were associated with an increase in impaired neurodevelopment in early childhood [95]. In a study with 137 children, mothers with high phthalate metabolites in urine during the third trimester gave birth to infants that were more susceptible to



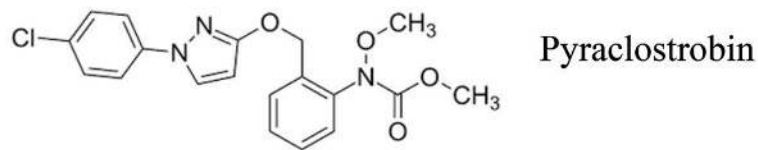
Famoxadone



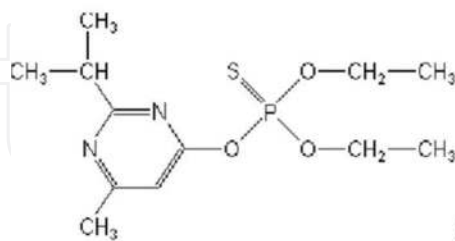
Fenamidonone



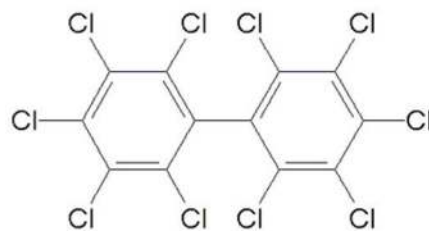
Trifloxystrobin



Pyraclostrobin



Organophosphorus



Chlorinated biphenyl

Figure 4. Structures of selected insecticides recorded as neurotoxin related to autism.

autism [96]. Larsson et al. have found a correlation between EDC and autism [97]. High serum phthalate concentrations have been recorded in autistic children [98]. Experimental animals show changes in development, synaptic organization, neurotransmitter synthesis and release, and brain structural organization due to exposure to EDC [99]. Cock et al. described links between brominated flame retardants, perfluorinated compounds, and ASD, yet additional weights of evidence are needed [100].

Several EDCs are known to disturb sex steroid and affect thyroid hormone (TH) levels which in turn are known to affect synaptogenesis, neuronal differentiation, migration, and myelination [101]. They play critical roles in brain development and potentially also for development of the connectome. TH receptor mutation in mice showed reduced density of GABAergic in the hippocampus, which was accompanied by more depressive and anxious behavior [102]. It is demonstrated that BPA inhibits the GABAAR-mediated response and that BPA affects development of GABAergic and dopaminergic systems [103]. Some studies indicate EDCs as a cause for neurodevelopmental disorders through GABAergic system changes [104, 105].

1.7. Radio frequency energy (RFE) of cell phone as neurotoxin in autism

During pregnancy, the possibility of fetal damage is increased as mothers are exposed to RFE [106]. Notably, the fetus may not be fully protected by amniotic fluid. It is well known that the pelvic structure permits deep penetration of the RFE to be absorbed within the developing fetus. Based on this, many investigations proposed that the dramatic increase in the incidence of autism since 1980 can be related to the neurotoxicity of cell phone radiation [107–109].

Based on our understanding on the etiological mechanisms in autism, such as oxidative stress, neuroinflammation, and glutamate excitotoxicity, the remarkable increase in the prevalence of autism about tenfolds since 1980 can be related to this dramatic increase which reach 1:45 on 2015 [110]. It is well documented that exposure to radio frequency radiation (RFR) can be accompanied by oxidative stress in human and animal models of autism [111, 112]. Unfortunately, these effects of cellular phone use can occur even at low and legal intensity which are now common environmental risk factors for infants, young children, adults, pregnant women, and fetuses. Cell phone radiations enhance free radical formation through the Fenton reaction as catalytic process through which iron converts hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is very potent and can induce damage of macromolecules, such as membrane phospholipids, DNA, and protein. Radio frequency radiation at very low intensities can also impair mitochondrial metabolism and modulate glutathione, glutamate, and GABA, which are substances related to the pathophysiology of autism [90, 113–116].

Fragopoulou et al. reported that through proteomic analysis of brain regulatory proteins from mice following prolonged exposure to electromotive force (EMF) led to either downregulation or overexpression of 143 proteins [117]. These altered proteins include neural function-related proteins, alpha-synuclein, glia maturation factor beta, cytoskeletal proteins, heat shock proteins, apolipoprotein E, as well as proteins of brain metabolism such as aspartate

aminotransferase and glutamate dehydrogenase. These authors pointed out that oxidative stress was consistent with some changes in proteomic markers. Alteration in blood and brain glutathione status and deficiencies of reduced glutathione are increasingly associated with autism. Fortunately, certain studies demonstrating that supplementation with antioxidants such as vitamins C and E reduced oxidative impacts on rat endometrium from due to vitamins E and C reduced adverse impacts on rat endometrium due to exposure to 900 MHz EMR [118]. Ilhan et al. proved that *Ginkgo biloba* has also prevented mobile phone-induced increases in lipid peroxides and nitric oxide levels in brain tissue as well as decreases in brain superoxide dismutase and glutathione peroxidase activities and increases in brain xanthine oxidase and adenosine deaminase activities, together with the relief of the histopathological cell injury [119]. **Figure 5** demonstrates the role of cell phone radiation in the etiology of autism through oxidative stress as a major etiological mechanism.

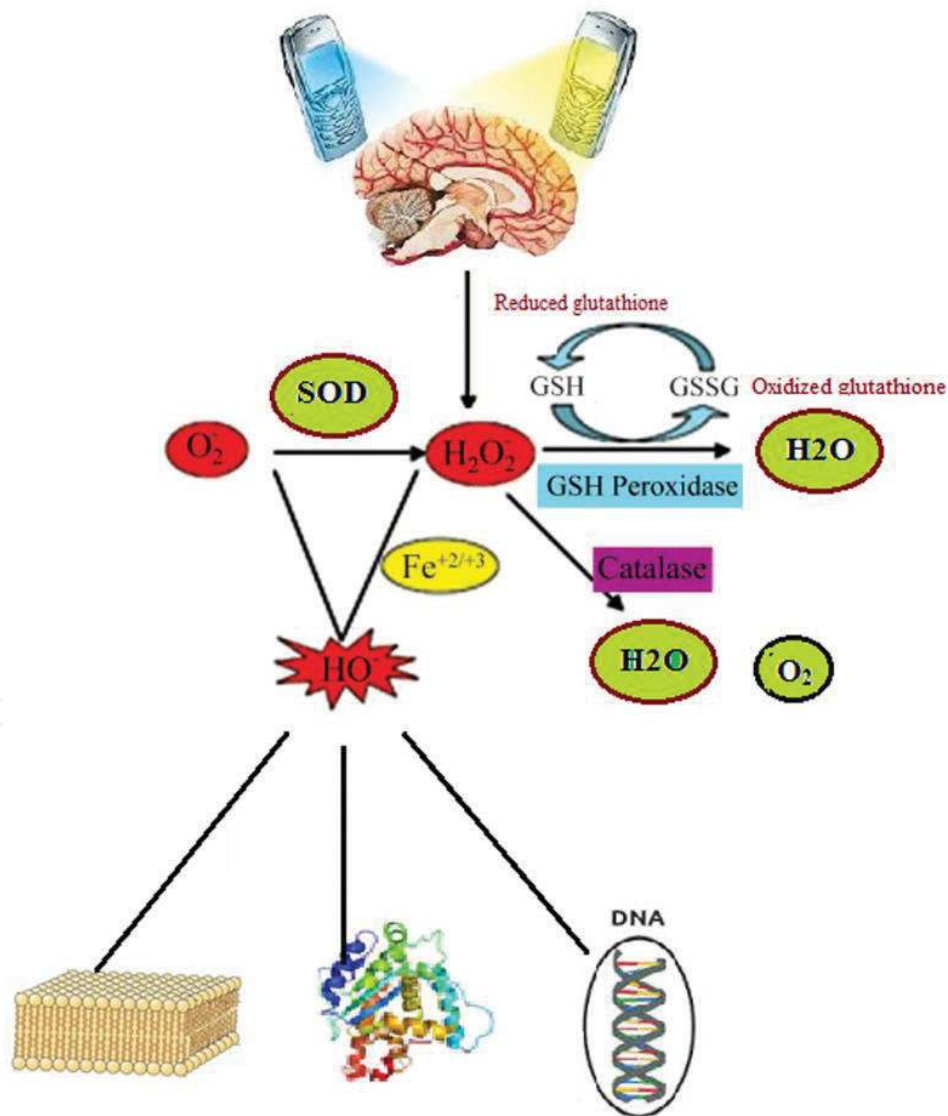


Figure 5. Role of cell phone radiation in the etiology of autism through oxidative stress as a major etiological mechanism.

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