# Alarming increase in the prevalence of autism: **Should we worry about pesticides?**

## Literature Review


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## DISCLAIMER

The following report is not intended to trivialize, denigrate or disrespect people with autism, who have equal merit as citizens in society, and whose social inclusion must be promoted and supported. This report's objective is to bring to light the impact that pesticide use can have on health and neuro-development prior to conception, in utero and during early childhood.

## LIST OF ACRONYMS

**ADHD: Attention deficit hyperactivity disorder**

**ASD: Autism spectrum disorders**

**CDC: Centers for Disease Control and Prevention**

**DDE: Dichlorodiphenyldichloroethylene**

**DDT: Dichlorodiphenyltrichloroethane**

**DNA: Deoxyribonucleic acid**

**EDC: Endocrine-disrupting chemical**

**GABA: Gamma aminobutyric acid**

**GSH: Gluthathione**

**PGE2: Prostaglandin E2**

**ROS: Reactive oxygen species**

**IRPeQ: Quebec Pesticide Risk Indicator**

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# 1 ALARMING INCREASE IN THE PREVALENCE OF AUTISM

Autism[1] is a complex neurodevelopmental disorder affecting 1 in 66 children in Canada and 1 in 64 children in the province of Quebec [3] (Figure 1). Between 2001 and 2017, the number of students with autism enrolled in Quebec public schools increased by 808% [4] (Figure 2). Autism is equally increasing at an alarming rate worldwide [1]. According to the Centers for Disease Control (CDC), autism has become *"an urgent public health concern"* [5] due to its rapidly increasing prevalence, which stands at 1 in 59 children in the United States.

Leading scientific researchers, medical experts, epidemiologists, child development specialists, and children's health advocates have raised the alarm that we are witnessing a "**pandemic of neurodevelopmental toxicity**" worldwide [6, 7]. Besides autism, developmental neurotoxicity may also be associated with attention deficit hyperactivity disorder (ADHD) as well as other more subtle and insidious cognitive deficits affecting a larger number of undiagnosed children, a worrying effect sometimes referred to as a "chemical brain drain" [6].

Autism impacts many areas of development in a child including communication, behaviour, social interaction, as well as sensory, motor and cognitive function. While each individual is unique and will have different needs, abilities and levels of intellectual functioning, many will however require significant support throughout their lives. Although persistent deficits in social communication and interaction; restricted and repetitive patterns of behaviour, interests or activities; inflexible adherence to routines; as well as hyper- or hypo-reactivity to sensory input are the benchmarks of the disorder [8], many other variables commonly accompany the diagnosis and add to the complexity of the disorder. These include, but are not limited to, the presence of associated intellectual disability, being non-verbal, lacking awareness of physical or social danger, wandering behaviours, hyperactivity, self-injurious behaviours such as head-banging, hand biting or scratching, as well as aggressive behaviours. A number of health problems are equally known to be associated with autism, such as gastrointestinal problems (i.e., constipation, digestive problems, reflux, restrictive eating), sleep disorders, epilepsy and allergies, but mental health conditions such as anxiety, depression, ADHD, and obsessive compulsive disorder can also be present [9].

1. For the purpose of making the following document easier to read, the word autism will be used to refer to all autism spectrum disorders (ASD) as well as to the previous terminology of *"pervasive developmental disorder"* 1. Dietert, R.R., J.M. Dietert, and J.C. DeWitt, Environmental risk factors for autism. Emerging health threats journal, 2011. 4(1): p. 7111. and Asperger's syndrome 2. Rosas, L.G. and B. Eskenazi, *Pesticides and child neurodevelopment*. Current opinion in pediatrics, 2008. 20(2): p. 191-197.



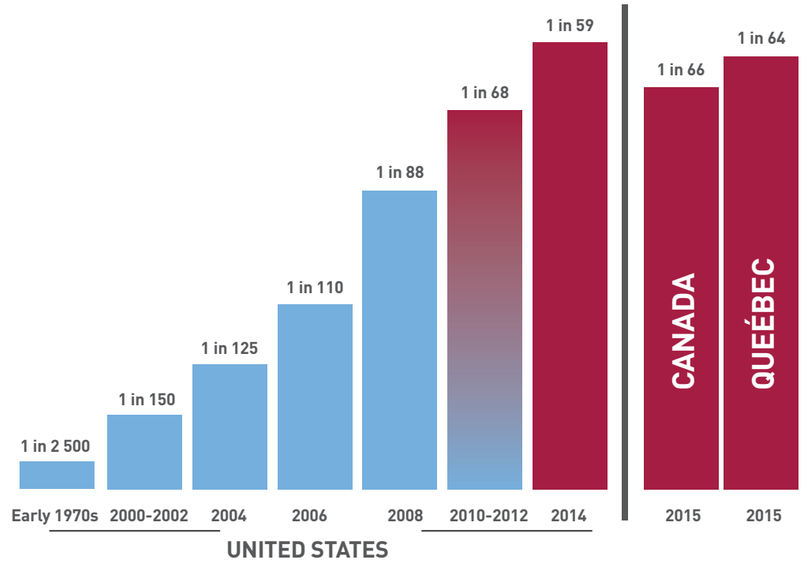



**FIGURE 1:** Increase in the prevalence of autism over time in the United States and latest statistics for Canada and the province of Quebec. Graphic compiled by authors based on statistics from CDC [10-12] and the Public Health Agency of Canada (PHAC) [13]. Statistics for Quebec and Canada are not available prior to 2015, according to PHAC.

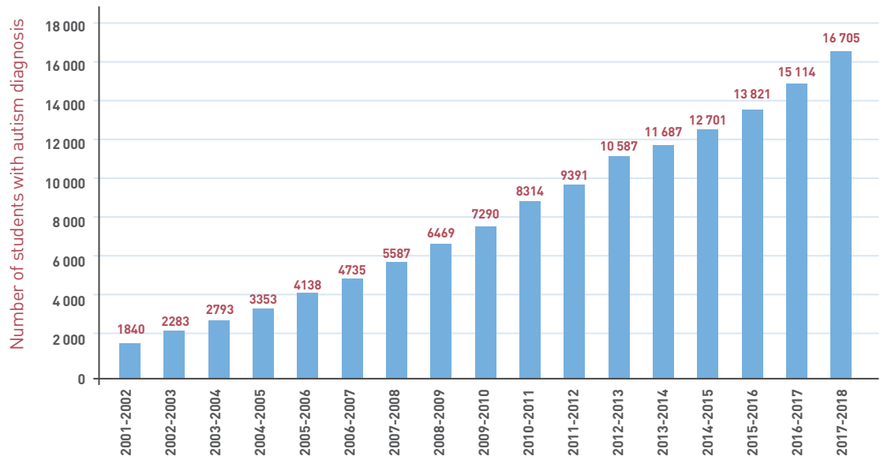
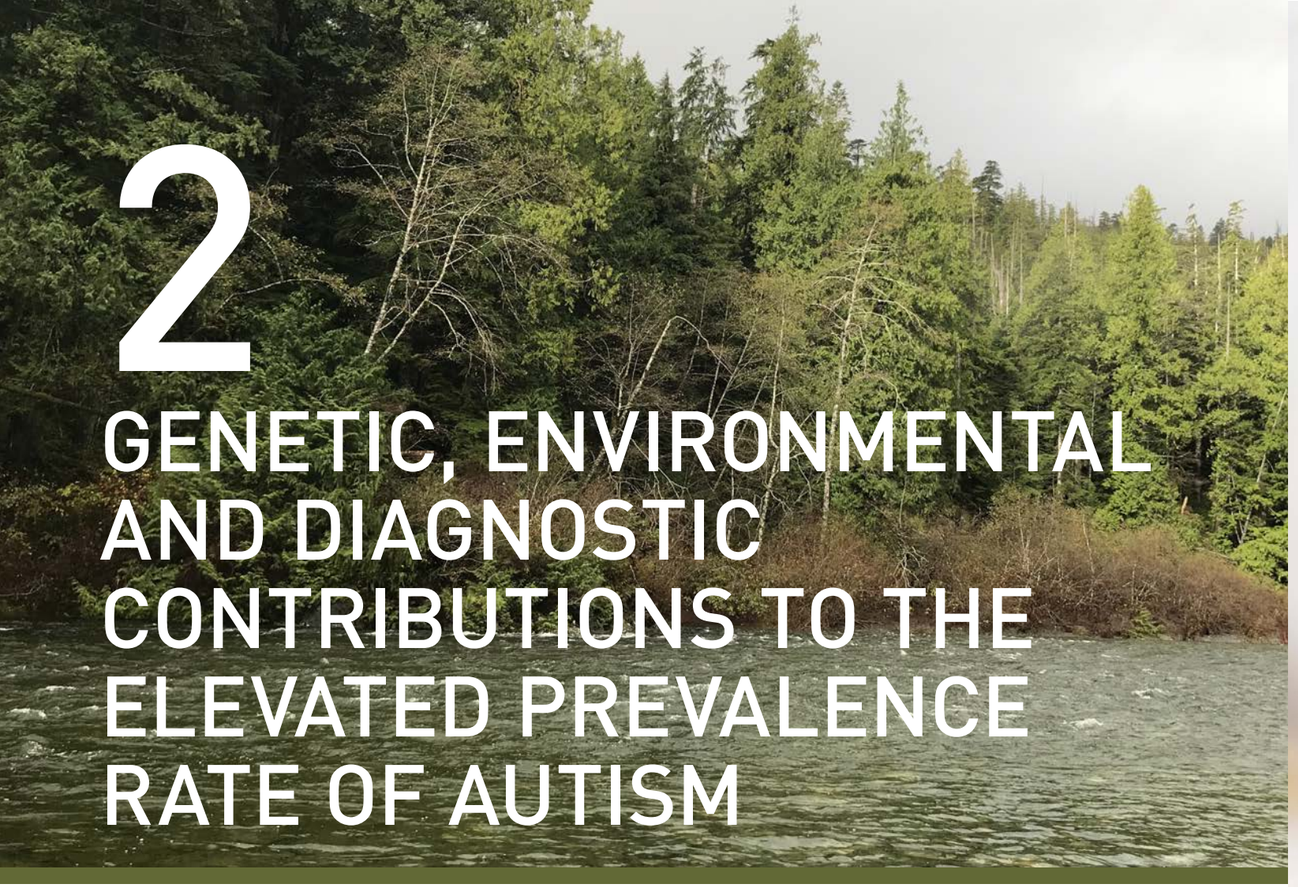



**FIGURE 2:** Increase in the number of students with an autism diagnosis from 2001 to 2017 in the province of Quebec. Graphic compiled by authors based on statistics from the Quebec Ministry of Education [4].



# 2 GENETIC, ENVIRONMENTAL AND DIAGNOSTIC CONTRIBUTIONS TO THE ELEVATED PREVALENCE RATE OF AUTISM

While autism is characterized by large phenotypic heterogeneity, it is likely that a myriad of biochemical pathways and processes culminate in an autism diagnosis [1]. It is generally agreed that the etiology stems from a complex interaction between genetic predisposition and environmental factors. Research indicates that **environmental factors play a much more important role than initially suspected** and that genetic vulnerability alone cannot account for the current increase being observed worldwide [6, 7, 14-21].

- Because genes evolve slowly, genetic factors alone could not explain the rapid increase in autism [18].
- While genetic vulnerabilities to autism do exist, there is no single gene associated with the development of autism [14, 15].
- Only around 10% of autism cases are related to other genetically-based neurodevelopmental disorders [10, 15].
- Changes to diagnostic criteria and greater awareness of autism may contribute to the rise in its prevalence, but neither can account for the current rapid growth being witnessed worldwide [18, 20]. These factors can only account for approximately 20% [22] to 33% [20] of the increase in autism prevalence.

Therefore there is a critical need to better understand the contribution of environmental factors.



# 3 IMPACT OF ENVIRONMENTAL TOXICANTS ON NEURODEVELOPMENT

While there are many environmental factors that may contribute to developing autism, no single environmental factor can independently fully explain the current autism epidemic (see Appendix 1). However **environmental toxicants are of particular concern** [1]**.** The association between exposure to environmental toxicants and autism is not new in research and it has been identified numerous times [1, 6, 7, 16-21, 23, 24]. The development of the central nervous system and the human brain is extraordinarily complex, and involves numerous time-sensitive events during gestation that are impacted by ongoing interactions between genetic and environmental factors [6, 18, 21]. There is a substantial body of research that indicates there are "complex interactions between genetic factors and certain environmental toxicants that act synergistically during critical periods of neurodevelopment in a manner that increases the likelihood of developing autism" [16, 25].

## 3.1 DEVELOPMENTAL VULNERABILITY DURING GESTATION

**The developing human brain is uniquely vulnerable to toxic chemical exposure, and major windows of developmental vulnerability occur particularly *in utero* but also during infancy and childhood as well** [6] (Figure 3). For instance, the blood-brain barrier and the placental barrier are not 100% effective at protecting the fetus against environmental toxicant exposure throughout pregnancy [18]. In addition, embryos, fetuses, newborns and children do not possess detoxification capacities at the levels demonstrated in adults, making them much more vulnerable to chemicals present in their environment [26, 27].

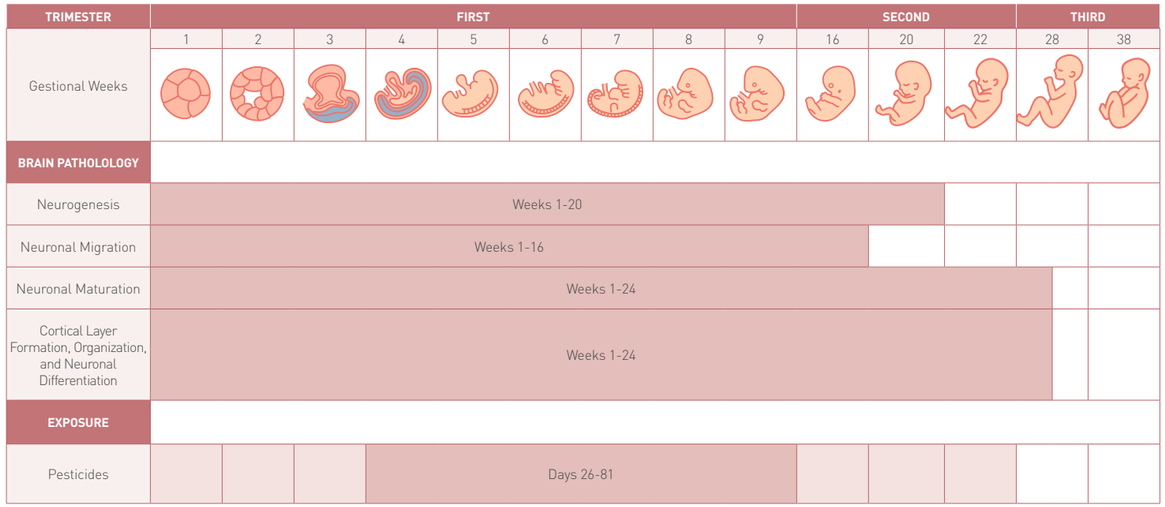



**FIGURE 3:** Critical periods of brain development and susceptibility to pesticides based on autism spectrum disorder studies [14]



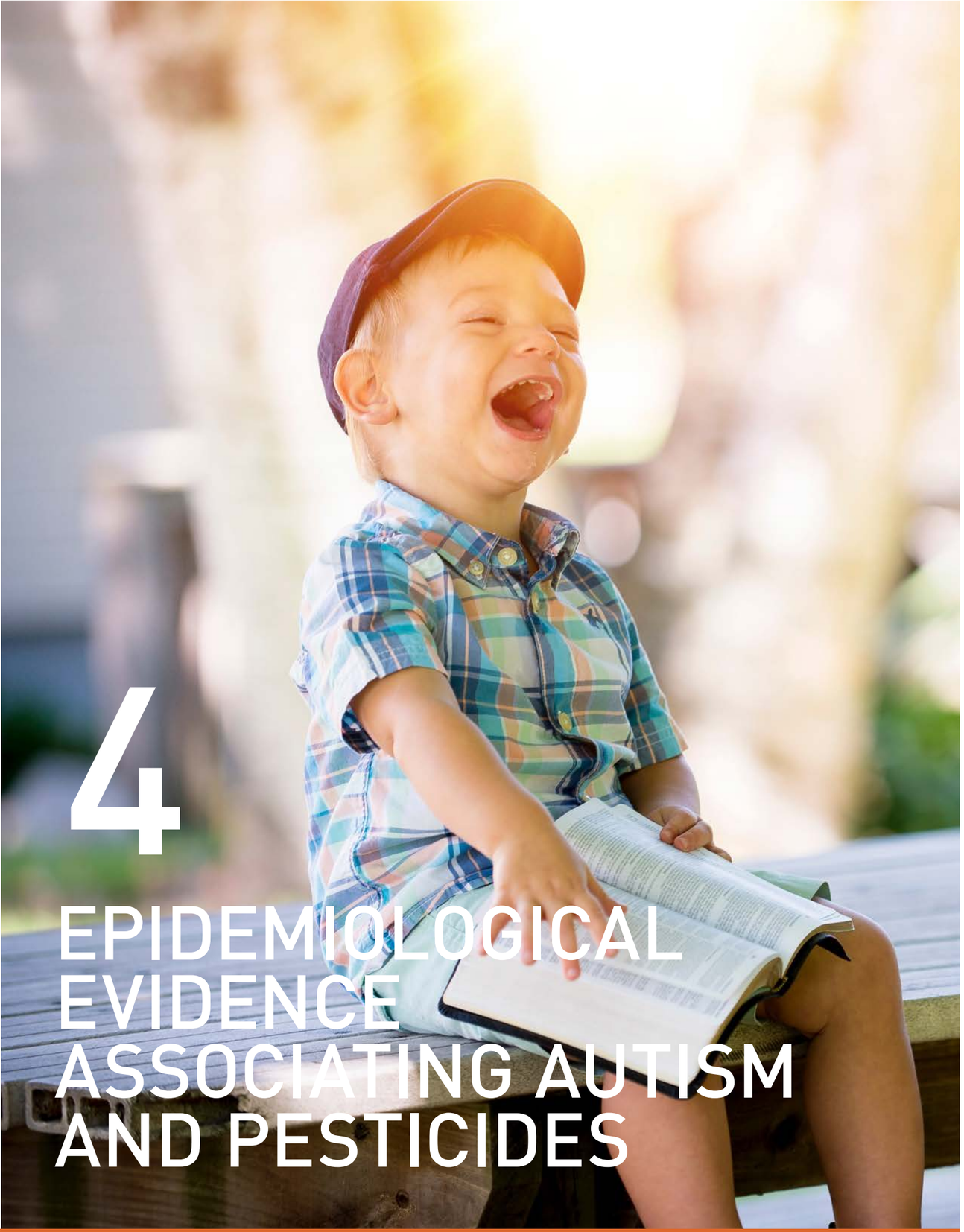
Furthermore, the human brain does not complete development in the womb, but pursues it into childhood, adolescence [28] and, potentially into adulthood [29]. **Research has shown that the developing human brain is extremely vulnerable to toxic chemical exposure, which can cause permanent damage at levels of exposure that would have little or no adverse effect in an adult** [6]. Consequently, it is extremely plausible that children are at an increased risk of neurodevelopmental disorders due to toxicant exposure [7, 19].

## 3.2 BIOCHEMICAL PATHWAYS AFFECTED BY TOXICANTS

There is a growing consensus that autism has a clear biological basis and should not solely be defined on the basis of behavioural observations [30], as it is currently the case [8]. Biological markers associated with autism include inflammation, oxidative stress, mitochondrial dysfunction and endocrine disruption. These physiopathological alterations are identified as important pathways in the development of autism [18, 26, 30]. In parallel, these same responses are also common consequences of exposure to environmental toxicants (see for instance inflammation [31], oxidative stress [32], mitochondrial dysfunction [33] and endocrine disruption [34]).

**Endocrine-disrupting chemicals (EDC) are known to contribute to cognitive deficits and neurodevelopmental disabilities** [35]. Appropriate levels of thyroid hormone is particularly important for normal brain development [35]. Prenatal exposure to a variety of maternal thyroid hormone-disrupting chemicals represents a plausible biological mechanism that can explain the current increase in neurodevelopmental disorders [18, 36], including autism and ADHD [35]. **The central nervous system is uniquely vulnerable and sensitive to adverse effects of thyroid-disrupting chemicals in the maternal and fetal environment during early development** [6, 18, 21, 35, 36]. **Maternal hypothyroidism increases the risk of autism four-fold** [37], so it is logical to examine associations between chemicals that affect signalling pathways of thyroid hormones and risk for autism in humans. While the association between gestational exposure to EDC and autism reported in epidemiological studies are common in the literature, there are some exceptions [37].

Numerous toxicants have been associated with autism including pesticides, phthalates, polychlorinated biphenyls (PCBs), solvents, air pollutants and heavy metals [1, 16, 38]. In one specific literature review, 92% of studies – that is, 34 out of 37 – reported an association between autism risk and environmental toxicant exposure [16]. In this same review, **the strongest associations between environmental toxicants and autism were reported for air pollutants and pesticides** [16]. Furthermore, **biomarker studies measuring toxicant concentration in blood or urine also reported an association between autism and levels of solvents, phthalates and, again, pesticides** [16].



# 4

# EPIDEMIOLOGICAL EVIDENCE ASSOCIATING AUTISM AND PESTICIDES

"Insecticides are designed to interfere with synaptic transmission in the central nervous system, however, neurotoxic properties of pesticides may have unintended consequences in the brain of developing children" [31]. **In epidemiological studies of environmental toxicants, pesticide exposure frequently appears associated with the development of autism** [1, 7, 16-20, 23, 24, 35, 36, 39]. Epidemiological studies examining pesticide exposure are either ecological in design (i.e., residential proximity to sites contaminated with pesticides) or biomarker-based (i.e., quantification of pesticides in body fluids), since voluntary testing of pesticides on pregnant women or babies would be considered unethical.

Epidemiological studies have identified that **residential proximity to agricultural pesticide application prior to conception and during pregnancy increases the risk of having a child with autism** [19] **as much as by 60%** (OR = 2.0; 95% CI: 1.1–3.6) [17]. Furthermore, the first biomarker-based evidence linking pesticide exposure in early pregnancy to autism measured maternal serum p,p'-DDE concentration (a metabolite of the insecticide DDT) [40]. **Offspring born from mothers who were most exposed to p,p'-DDE** (75th percentile exposure group) **showed a 32% increase in the risk of autism** (OR = 1.3; 95% CI: 1.0–1.7). Furthermore, **the odds of having both autism and intellectual disability were increased by more than two-fold in this high-exposure group** (OR = 2.2; 95% CI=1.3–3.7) [40].

In both types of epidemiological studies, when considering estimated or measured exposure during the period of gestation, **pesticides are amongst the toxicants with the highest association with autism** [16, 17]. This is not surprising, since pregnancy is a period in which there is very rapid development of the fetus' central nervous system. Some studies report a two- to five-fold increase in the odds ratio of autism with gestational exposure to pesticides [16]. Pesticides are of great concern, because research now suggests that **exposure to pesticides, whether inside or outside the home, even prior to becoming pregnant, can also affect the neurodevelopmental outcome of future offspring that has not yet been conceived** [7, 17]. More recent research even indicates that while prenatal pesticide exposure contributes the most to the risk of developing autism [10], **pesticide exposure in early infancy also increases by 50% the risk for a child with autism to have comorbid intellectual disability** [41]. However, the evidence linking pesticide exposure to autism is stronger during the gestational period than for childhood exposure [16]. **A study by Roberts et al. also demonstrated a dose-response for autism risk with organochlorine exposure: the odds ratios of autism increased monotonically with greater exposures to organochlorines** [21, 23, 41, 42].

**Furthermore, many pesticides, particularly neurotoxic insecticides** [17]**, have not been tested for their neurodevelopmental toxicity** [6] **during their registration process and are known to be endocrine-disrupting chemicals**.

## 4.1 SPECIFIC PESTICIDES ASSOCIATED WITH AUTISM

Scientific literature reports that many pesticides are associated with autism, and this even at low-level exposures considered to be below toxic range [43]. Insecticides that act on the nervous system are therefore obvious research targets for their potential roles in autism. Insecticide chemical families associated with autism include carbamates [17], organochlorines [21, 43], organophosphates [17, 21, 41, 44, 45], pyrethroids [17, 21, 41], neonicotinoids [46], organobromine [41] and macrocyclic lactone derivatives [41]. Organochlorines, organophosphates, and pyrethroid pesticides all have an effect on developing synapses (the connections between neurons)

and this may explain associations with neurodevelopmental disorders [47]. Among the insecticides that have been identified to be associated with autism are DDT and its derivative DDE [40], chlorpyrifos [17, 41, 43, 44], diazinon [41, 43], malathion [41], avermectin [41], permethrin [41], cypermethrin [43], bifenthrin [44], imidacloprid [46], dicofol [18, 43], endosulfan [18, 43], and methyl bromide [41]. Beside insecticides, herbicides (glyphosate [41, 48], and ammonium glufosinate [49, 50]), fungicides (myclobutanil [41], phosphine [51], pyraclostrobin, trifloxystrobin, famoxadone and fenamidone [52]) and rodenticides (rotenone [52]) have also been listed in studies showing correlations with autism. **Many of these pesticides are still registered for sale in Canada according to the Pest Management Regulatory Agency of Health Canada** (Table 1). For more details on the history of pesticides, epidemiological or mechanistic evidence linking specific pesticides to autism, please refer to Appendix 2. For more details on the type of epidemiological study and exposure assessments on various primary studies, please refer to Appendix 3.

**TABLE 1:** Various pesticides that have been mentioned in studies exploring associations with autism and their registration status in Canada.

| PESTICIDE CATEGORY | CHEMICAL FAMILY | ACTIVE INGREDIENT | REFERENCE | PRODUCTS REGISTERED IN CANADA[1] |
|---|---|---|---|---|
| Insecticides | Carbamates | | [17] | 76 |
| | Organochlorines | | [21, 43] | |
| | | DDT and DDE (metabolite) | [40] | 0 |
| | | Dicofol | [18, 43] | 2 |
| | | Endosulfan | [18, 43] | 0 |
| | Organophosphates | | [17, 21, 44, 45] | 12 |
| | | Chlorpyrifos | [17, 41, 43, 44] | 24 |
| | | Diazinon | [41, 43] | 9 |
| | | Malathion | [41] | 32 |
| | Pyrethroids | | [17, 21] | |
| | | Permethrin | [41] | 345 |
| | | Cypermethrin | [43] | 9 |
| | | Bifenthrin | [44] | 3 |
| | Neonicotinoids | Imidacloprid | [46] | 65 |
| | Organobromines | Methyl Bromide | [41] | 5 |
| Herbicides | Phosphonic acids/ organophosphorous | Glyphosate | [22, 41, 48, 53-56] | 448 |
| | | Glufosinate ammonium | [49, 50, 57] | 60 |
| Fungicides | Inorganics | Phosphine | [51] | 27 |
| | Strobilurines | Pyraclostrobin | [52] | 5 |
| | | Trifloxystrobin | [52] | 1 |
| | Oxazolidinediones | Famoxadone | [52] | 2 |
| | Imidazolones | Fenamidone | [52] | 1 |
| | Triazoles | Myclobutanil | [41] | 4 |
| Insecticides | Chromenones | Rotenone | [52] | 4 |

Notes: [1]The number of products registered in Canada is based on a search on Health Canada's Pesticide Management Regulatory Agency pesticide labels database in June 2019 [58].



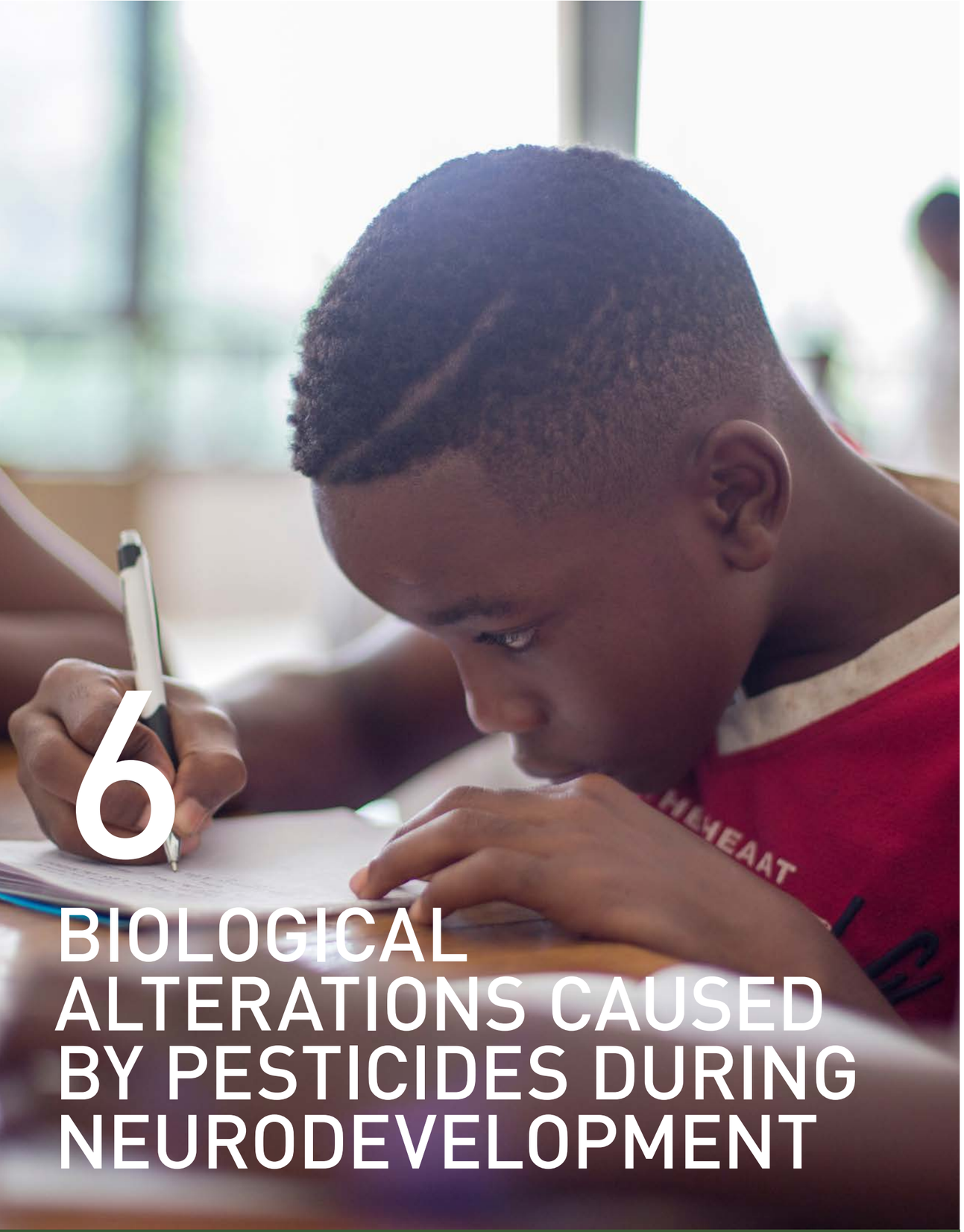
Furthermore, the succession of pesticides entering the market, and later restricted or completely banned, reveals the worrying process of regrettable substitutions [59, 60]. Exceptional authorizations granted for the use of otherwise banned pesticides are also worrying observers due to their high frequency [61]. This story is also marked by "legacy pesticides" that were banned, but remained present in the environment, in our food and water due to their high persistence over time (i.e., DDT, although banned since the early 1970s, remains in the environment for hundreds of years). **The most worrying classes of pesticides associated with autism are insecticides, which are known to target the nervous system. Unfortunately, advanced neurodevelopmental testing in the mandatory registration testing of many pesticides is lacking** [60]. As such, if an effect is not obvious through mandatory acute testing, it may very well remain unstudied. This applies particularly to any long-term effects on human health associated with pesticide use in real life (as opposed to mandatory testing done in controlled environments before registration). This is likely the case with neurodevelopmental disorders such as autism. As society substituted organochlorines with organophosphates, then pyrethroids and more recently neonicotinoids, we left a legacy of health issues that can transcend generations and lead to yet unidentified consequences.

# 5 ROUTES OF PESTICIDE EXPOSURE

**Other than overt exposure to pesticides when actually applied in one's indoor or outdoor environment, the general population is also exposed to pesticides or their metabolites through drinking water, food, air pollution, dust, insect repellent, and pet flea and tick treatments** [19, 23, 45, 62-65]. For children, the primary route of exposure to many pesticides, for instance permethrin, is ingestion of contaminated food [66], while organic diets have been shown to decrease pesticide loads in children [67], both in rural and urban locations [68]. Domestic exposure through air and dust is equally important in homes where pesticides have been used or in homes of farmers [69]. A non-negligible alternate route of exposure for children and spouses of farmers may also be exposure through contact with clothes or surfaces [69, 70].

Exposure to pesticides is nearly ubiquitous. For example, most US households have measurable levels of insecticides (as detected in floor wipe samples); the most commonly detected is permethrin (89%) [71]. Cypermethrin (46%) is also commonly detected [71, 72]. Low-income populations are also greatly at risk: permethrin was detected in all (100%) urban public housing units surveyed in Boston (Mass., USA), while cypermethrin had a prevalence of more than 90% [73]. Dust concentrations are a useful indicator for adult and children exposure to pesticides [69]. The ubiquity of pesticides in our environment unsurprisingly results in pesticide and metabolite detection in nearly all children. For example, a Quebec study from 2004 also showed that 98.7% of 442 urine samples taken from children age 3 to 7 had the presence of organophosphate pesticide residues [74]. Another study in California revealed that the majority of children (>72%) have pesticide metabolites in their urine, including residues of organophosphorous and pyrethroids insecticides [68], which are associated with autism. In China, 100% of children and adults tested in rural areas had imidacloprid residues in their urines, and this detection was of 95% in urban subjects [75]. A literature review of glyphosate concentrations in urine revealed frequencies above analytical limits of detection fluctuate between 30% and 80% amongst seven studies, but dismissed potential health effects as reported concentrations were below the acceptable daily intake (ADI) or the acceptable operator exposure level (AOEL) [76] (even though advanced neurodevelopmental toxicity studies were never required, therefore not considered in glyphosate registration [77]). One of these studies included in the literature review was conducted in the USA with subjects specifically avoiding genetically modified food and pesticides. Glyphosate residues were still found in the mother's milk of 30% of the 10 subjects tested and in the urine of 37% of the 35 adults and children tested [78].

The fact that many pesticides and their breakdown products have a low rate of biodegradability may lead to accumulation in the environment [79]. This implies that the harm they are likely to cause in exposed adults and children may extend long after we stop using them.



# 6 BIOLOGICAL ALTERATIONS CAUSED BY PESTICIDES DURING NEURODEVELOPMENT

## 6.1 PESTICIDE IMPACT ON CHILD NEURODEVELOPMENT

Many pesticides are well known for their endocrine-disrupting capacity (such as thyroid function), as well as for their ability to disrupt neurodevelopmental signalling [18, 35]. Interference with normal hormonal signalling during brain development may have significant impacts on the unborn child. For example, during the first 10–12 weeks of gestation, the fetus is fully dependent on the functioning of his/her mother's thyroid gland [36]. Thyroid hormone is essential for normal brain development because "it influences during specific temporal windows, neurogenesis, neuronal migration, neuronal and glial cell differentiation, myelination and synaptogenesis" [36]. As such, adequate maternal thyroid function plays an essential role in fetal brain development and function [18, 35, 44]. **Research indicates that maternal hypothyroidism increases autism risk four-fold [35] and even moderate forms of maternal thyroid dysfunction may affect child cognitive development and increase neurodevelopmental disorders** [36]. It is suspected that the development of autism begins in the 8th to 14th week of gestation [15].

**Studies directed at pregnant women found that those with higher levels of pesticides in the umbilical cord plasma or in serum samples had a greater risk of bearing a child who would develop autism** [14, 18, 38]. **During gestation, the blood brain barrier and the placental barrier transport essential nutrients between the mother and fetus and into the central nervous system of the fetus** [18]. **However, certain pesticides are capable of crossing these barriers** using certain transporters normally carrying other signalling molecules, such as prostaglandin E2 (PGE2) [18]. Therefore, these pesticides can interfere with various biochemical signalling processes of the developing brain and body of the fetus [18]. Prenatal development of the brain requires highly specific signalling from key biological pathways that carefully regulate the expression of genes. These pathways can be turned on or off, or expressed in specific concentration gradients, during different stages of development [18].

**Pesticides also influence neurodevelopment by disrupting endocrine function, such as PGE2 signalling, which is essential in the development and functioning of the brain** [18]**. Pesticides can also cause mitochondrial dysfunction and disrupt neuronal signalling (acetylcholine, serotonin, GABA, sodium and calcium channels)** [16, 17, 39, 44]. For example, acetylcholine is a neurotransmitter specifically targeted by a number of pesticides. It normally plays a critical role in learning, attention, and memory and its concentration is known to be reduced in frontal and parietal cortices of children with autism.

## 6.2 METABOLIC AND BIOCHEMICAL INTERFERENCES OF PESTICIDES AND AUTISM

**Pesticide exposure may lead to numerous biochemical interferences**, including immune dysregulation, hormonal aberrations, nutrient deficiencies, epigenetic alterations and/or by inducing *de novo* DNA changes [15, 16]. Pesticides can also induce oxidative stress, which leads to the production of reactive oxygen species (ROS) that can decrease mitochondrial function (involved in cellular respiration). Decreased mitochondrial function is a recurring trait in autism [18]. Some people with autism have physiological abnormalities involving oxidative stress and lower glutathione (GSH) levels [16]. Oxidative stress profoundly alters various biological structures, such as cellular membranes, lipids, proteins and nucleic acids, and it is involved in numerous malignancies [80].

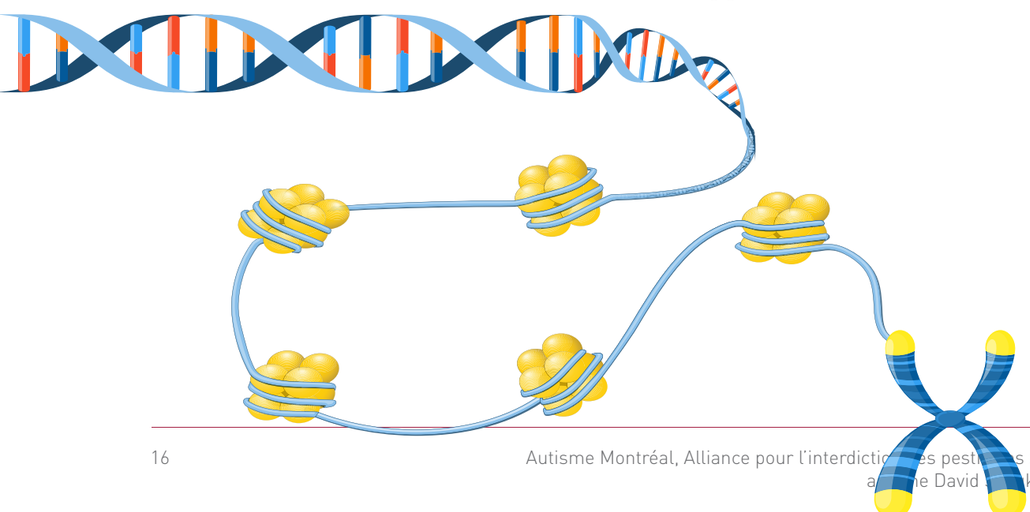
Glutathione is one of the most important scavengers of ROS, which protects the body against oxidative stress. Males, who are predominantly affected by autism (4:1 ratio), are known to have heightened oxidative stress, lower GSH levels and are more susceptible to the effects of various toxicants, including pesticides [16].

This raises the possibility that children with autism may be more vulnerable to synergistic actions on biochemical pathways that are simultaneously damaged by toxicants and due to a reduced detoxification capacity. Indeed, studies have shown that the *PON1* gene, an environmental response gene critical for detoxification of pesticides, exhibits polymorphism in people with autism [16]. Children with autism may have less active paraoxonases, the enzyme responsible for organophosphate degradation, making them more sensitive to this family of insecticides [81]. Therefore, **children with autism might metabolize toxicants differently and may experience toxicity to pollutants at a lower concentration compared with typically developing children** [16, 39].

Some pesticides, including glyphosate, also interfere with the "shikimate pathway" in human gut microbes, production of neurotransmitter production such as serotonin, and sulfate synthesis, which increases a number of biomarkers (p-cresol, serum sulfate deficiency) that are related to autism [15].

Several studies have shown that imbalances of excitatory/inhibitory neurotransmitters resulting from neurodevelopmental impairments in glutamatergic and GABAergic system might be a common pathological mechanism for autism and ADHD [82]. GABA (gamma aminobutyric acid) is an inhibitory neurotransmitter essential and critical for proper development and functioning of the brain [82]. Autism has been associated with alteration of GABAergic pathways either through genetic variations or neuronal interaction modulation that affect the excitatory-inhibitory ratio of the brain [16]. Numerous pesticides, such as pyrethroids, are equally known to interfere with GABA signalling [14].

DNA methylation, a process by which methyl groups are added to the DNA molecule, is a crucial epigenetic process that leads to different expression of the DNA genes. Methylation is a key element amongst the control mechanisms that govern gene expression in vertebrates [83]. DNA methylation may also play a role in the inheritance of certain traits [84]. **Research indicates that pesticides professionally applied outside or inside homes could alter placental DNA methylation more than other factors** [19]**. Thus, pesticides may be interfering with multiple biochemical pathways simultaneously with epigenetic ones in the developing fetus and placenta. Epigenetic mutations may thus simply be co-occurring secondary effects of mutagenic agents to which a developing fetus is exposed to during gestation** [15].








# 7 OTHER HEALTH IMPACTS OF PESTICIDES

Exposure to pesticides is known to cause a range of health and developmental concerns other than autism. Pesticide exposure is known to cause structural abnormalities of the brain, low head circumference, and persistent neurobehavioural deficits [6]. These include loss of IQ points and associated intellectual disability, ADHD, impaired cognition and motor function, reproductive problems for males and females, childhood and adult obesity, diabetes [35, 36, 85], as well as childhood leukemia [86]. Therefore, children are at an increased risk for many health consequences as a result of being exposed to pesticides. Furthermore, **pesticide exposure of parents and grandparents can be linked to health consequences in unexposed children or grandchildren**. This is the case for grandparent exposure to the insecticide DDT, which is associated with obesity in their grandchildren, theoretically due to epigenetic factors such as DNA methylation [87]. Finally, since many pesticides are highly persistent in the environment [79], they can therefore impact health long after their application or their restriction.

# 8

# SOCIETY'S ROLE IN PROTECTING FUTURE GENERATIONS

The long-term adverse effects of pesticides have been described as a "silent pandemic" [88]. The resulting neurodevelopmental disabilities are at a cost that is far too high for the affected individuals, their families, their communities and society as a whole [1, 35]. These costs include the health care system, physician's fees, specialized educational programs, specialized residential services, welfare and employment support [1]. A 2014 Canadian report on the financial costs associated with supporting a person with autism estimated that the lifespan value of caregiver time alone to support a severely affected individual with autism to be approximately $5.5 million above the costs of a neurotypical individual. In cases where adolescents and adults with severe autism are placed into long-term care or other supported housing arrangements, the annual cost of housing, which includes caregiver time, can be $400 per day, amounting to approximately $150,000 a year [89]. US statistics from 2011 reported that the total costs per year for children with autism in the United States were estimated to be between $11.5 billion and $60.9 billion. This significant economic burden represents a variety of direct and indirect costs, from medical care to special education to lost parental productivity [7] [90]. Another report from the US pertaining to 2015 indicated that the cost of caring for Americans with autism had reached $268 billion in 2015 and would rise to $461 billion by 2025 in the absence of more effective interventions and support across the lifespan [91].

Families may suffer from a net income loss of approximately 14% as well as experience problems that affect their capacity to work [1]. Families and particularly parents experience additional stress and psychological challenges related to the imperative to reorganize their entire daily functioning around their child's needs [1].

The need to protect future generations of children and families is critical. The obvious question that begs to be asked is, **what is being done to protect future fetuses and future mothers from pesticide exposure** [19]?

**Our society, our governing leaders and our public health agencies must take action to reduce widespread exposure to chemicals that interfere with fetal brain development** [7]. Given that the scientific literature consistently reports an association between *in utero* pesticide exposure and impaired child neurodevelopment, clinicians should educate parents, pregnant couples and potential parents about prevention strategies to avoid exposure due to the potential impacts on child neurodevelopment. Policy-makers should act accordingly [2,7].

**Since non-genetic factors influence the underlying neurodevelopmental processes during the prenatal period, it is essential to act upon the modifiable preconception and/or prenatal factors that have been associated with autism** [16]. By increasing awareness and knowledge about the potential adverse effects of pesticides on fetuses, children and future mothers, future generations stand to potentially have healthier brain development and overall health. Autism is one condition amongst a very large number of chronic conditions that have followed similar rates of increasing prevalence. These include asthma, type 1 diabetes, metabolic syndrome, schizophrenia, sleep disorders, etc. [1]. These conditions all have early life origins, can be connected to a grid of comorbidities and have environmental factors that remain to be precisely identified in future epidemiological research [1]. "An effective environmental risk reduction strategy for autism is likely to have a broader relevance that could encompass other developmentally based diseases." [1]

While research indicates taking higher doses of folic acid supplements prior to conception and during early gestation may mitigate some of the risk of a fetus developing autism when exposed to pesticides during pregnancy [14], it does not completely eliminate it [24].

Thus, **decreasing exposure to harmful chemicals such as pesticides is an essential step for society to take**. Facing the current pandemic of neurodevelopmental toxicity, action can and must be taken without further delay to limit exposure to pesticides (35).

# 9 POLICY RECOMMENDATIONS

The responsibility to curtail neurodevelopmental toxicity depends on what collective actions will be taken [6]. **To achieve such an endeavour, a coordinated and concerted action plan containing comprehensive and concrete strategies must be quickly developed and implemented by provincial and federal governments in Canada, as well as internationally.** Here are a few of these strategies that should guide this effort in Canada:

## 9.1 FEDERAL JURISDICTION

- Cancel or restrict registration for all known neurodevelopmental toxicants and endocrine disruptors, whether or not they have carcinogenic properties.

- Reform the *Canadian Environmental Protection Act* and *Pest Control Products Act*, particularly the registration and reassessment process of pesticides must be based on independent scientific studies rather than industry-funded studies, and should include:

  - a thorough evaluation of complete formulations rather than focusing on the active ingredients

  - assessment of long-term effects on human health and environment

  - advanced neurodevelopmental studies for any pesticide that may interfere with neurotransmitters or endocrine signalling

  - cumulative and synergistic effects in their risk assessment according to the pesticide field uses and environmental co-occurrence of pesticides

- Health Canada must decrease the maximum "safety residue limits" of pesticides allowed in food [45], water and air to levels that are safe for vulnerable populations, such as pregnant women and children.

- Assess and identify alternatives to toxic substances and place the burden on industry to show that safer substitutes are not otherwise available.

- Mandatory integrated pest management and promotion of non-toxic or least toxic control methods to address pest problems for both residential and agricultural practices [45].

- Mandatory warning labels with easily recognizable symbols and colours placed on toxic products sold to the general public.

- Ban advertisement of pesticides in all media.

- Ban any form of discounts on pesticide products.

- Prohibit pesticide product placement in high-traffic areas (store entrance or exit, near cash register, etc.).

- Not allow pesticides to be sold in supermarkets or drug stores.

- Obligate companies that develop pesticides to provide funds for ongoing independent scientific research on the impact of pesticides on human health and the environment, using a governance structure that prevents any form of conflict of interest.

## 9.2 PROVINCIAL JURISDICTION

- Restrict or completely ban the use of pesticides known as neurodevelopmental toxicants or endocrine disruptors, whether or not they have carcinogenic properties.

- Reform and update the Quebec Pesticide Risk Indicator (QPRI/ more commonly known by its French acronym IRPeQ). This indicator must be based on independent and up-to-date science.

- Put in place a mandatory digital system for tracking and reporting all pesticide application, not only for select top-ranked pesticides as is currently the case.

- Improve the mandatory reporting systems for retail and bulk sales to include a mandatory digital information transmission, enabling regionalized maps of pesticide sales, describing their exact nature and volume. In France and California, such mapping has shown to be a useful tool for strengthening epidemiological research on pesticides.

- Organize prevention and awareness campaigns by public health agencies [36] to protect the health and development of future generations from pesticide-related risks.

- Implement a comprehensive strategy to raise awareness and train health professionals, including general physicians, obstetricians, pediatricians, psychologists, child development specialists, child therapists as well as nurses, on the impact of environmental toxins such as pesticides on fetuses. Expand training if possible to all paramedical professionals, for example midwives whose mission is preventative care and follow-up of pregnant women.

- Implement mandatory training for farmers, professional gardeners as well as exterminators, on the potential impacts of pesticides on health.

- Quickly implement economic disincentives, such as an additional environmental tax, when purchasing or applying pesticides, especially for agricultural use. This environmental tax could be reinvested for the purpose of developing organic farming strategies.

- Provide further financial incentives to farmers to facilitate transitioning their farming practices into organic farming practices.

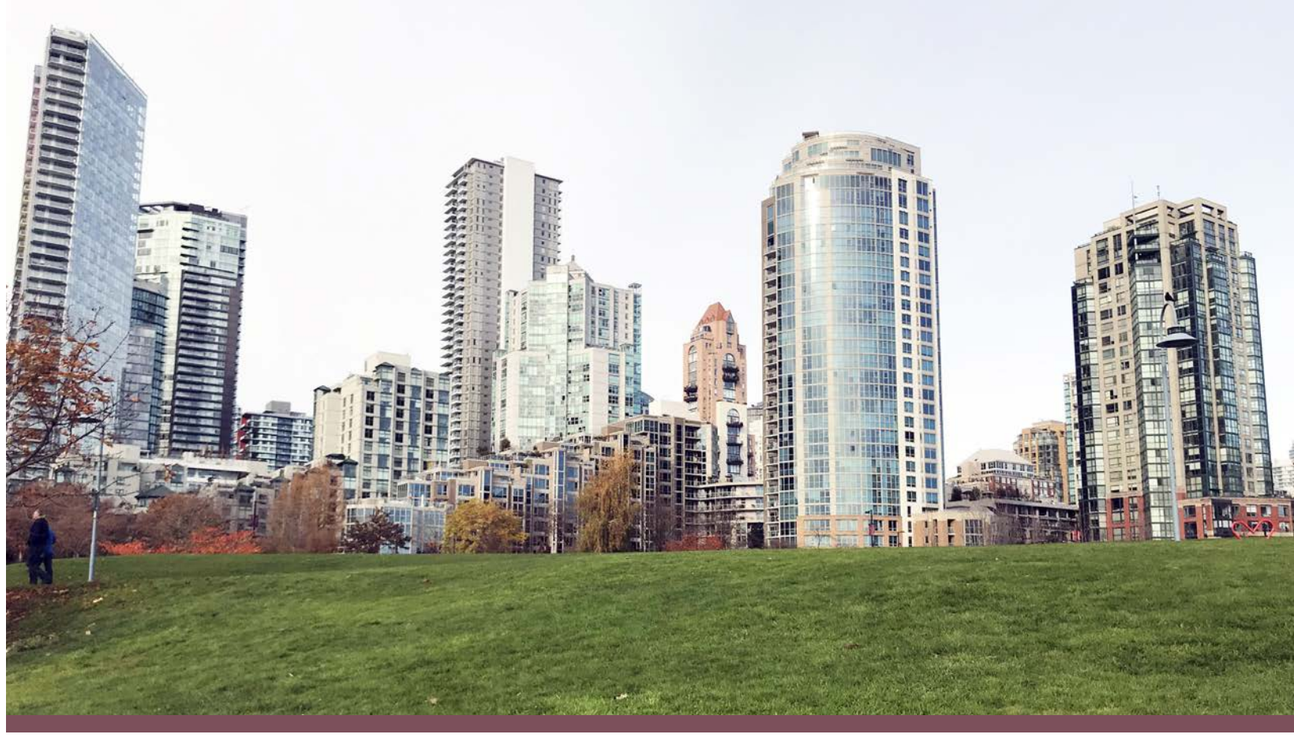
- In urban areas, further restrict the use of pesticides inside and outside residential homes and buildings, particularly in health care facilities, schools and daycares.

- In agricultural areas, restrict the use of pesticides near homes, buildings and public roads. Research has shown detrimental health effects within a radius of 2 km [17].

- Require a public announcement to be made in advance of pesticide application on agricultural fields (also taking into consideration wind direction) in order to help minimize unnecessary exposure from pesticide vapour drift. Awareness campaigns should also include strategies to protect children and homes, such as closing windows and A/C systems, not allowing children to play outdoors during or after application, etc.

- Impose high fines to companies and individuals that violate regulations/bylaws.





## 9.3 MUNICIPAL JURISDICTION

- Prohibit cosmetic pesticide use outside of homes in municipalities that do not yet have such a bylaw.

- Prohibit pesticide sale in municipalities where bylaws restrict their use.

- To gain public support and frame pesticide reduction with a positive messaging, adhere to certification programs that promote biodiversity conservation and pesticide use reduction. See for example, Monarch-Friendly City program from the David Suzuki Foundation.

- Conduct educational and awareness campaigns, which should:

  - Discourage indoor pesticide use by providing information to citizens and companies on the risks of pesticides to humans and the environment.

  - Improve basic insect knowledge, which can increase public tolerance of insects that do not pose health or structural issues to buildings.

  - Promote behaviours and actions that minimize the risks of home infestations such as preventing insect entry (sealing cracks or openings in homes), reduce attractiveness to insect pests (improve food and garbage storage), reduce available food and water sources for insect pests (improve house cleanup and residents' behaviours) and reduce the risk of actively transporting insects, such as bed bugs, inside homes (by inspecting furniture or plants before entry, and putting luggage in quarantine after a trip).

# 10

# CONCLUSION

The ramifications of pesticide exposure are potentially devastating to unborn fetuses, and very young children, with a large range of neurodevelopmental risks, making pesticide exposure a public health challenge. **While pesticides are one class of the many potential environmental toxicants associated with the development of autism, it is one that can and must be collectively acted upon to protect early brain development** [41]. Society can no longer afford to turn a blind eye to this issue. The risks and the costs involved are just too high. The motivations for approving the use of pesticides must not and cannot be to the detriment of public health and safety. Collective and proactive efforts must be undertaken to limit human exposure to pesticides particularly during critical periods of life, such as during pregnancy and early childhood. Awareness of the impact of pesticide exposure should be expanded through dissemination of information and prevention strategies involving multiple health professionals and government bodies.

## METHODOLOGY

This literature review was conducted between October 2017 and July 2019 using the academic search engine Google Scholar, and various key words including ''pesticide'', ''autism'', ''autism spectrum disorder'', ''organochlorines'', ''organophosphates'', ''pyrethrinoids'', ''glyphosate'', ''DDT'', etc. Primary research articles detailing laboratory analysis on test animals, mechanistic studies and epidemiological studies were considered. Literature reviews were also included though the original research papers were quoted where necessary and when accessible. A total of 158 studies were included in the current literature review. This review was not meant to be exhaustive and is not a meta analysis of published articles. However, no study failing to report an association between pesticides and autism were intentionally rejected: these results were cited as negative. The goal of this literature review is to raise awareness on the abundance of the scientific literature which associates pesticides with autism, to encourage societal decisions based on the precautionary principle despite current limits of current knowledge in order to prevent further harm, and to encourage further research to better elucidate potential modes of action and explore if correlations also imply causation.

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# APPENDICES

# APPENDIX 1

## A non-exhaustive list of environmental risk factors involved in the development of autism

| RISK FACTORS | COMMENTS | REFERENCE |
|---|---|---|
| **Maternal immune activation** | Maternal infections, allergies, asthma, stress, fever and inflammation during pregnancy are associated with the risk and severity of autism. Bacterial infections, like streptococcus could activate the maternal-fetal immune system, triggering autistic-like phenotypes. | [92-98] |
| **Complications during pregnancy or at birth** | Prenatal and perinatal factors (i.e., preeclampsia, polyhydramnios, oligoamnios, placenta praevia, umbilical cord knot, gestational diabetes, lack of oxygen) are associated with the severity of autism symptoms. There is a very high risk for developing autism in extremely preterm-born children. | [99-103] |
| **Maternal hormonal imbalances** | A prenatal sex steroid theory of autism was formulated on the basis of associations between maternal hormonal imbalances and autism in offspring. Elevated levels of many hormones (androgens, progesterone, cortisol) in the amniotic fluid are associated with autism. Conditions that may elevate androgen, for instance polycystic ovary syndrome, are associated with a higher rate of autistic traits in mothers and also in their offspring. | [104] |
| **Use of acetaminophen (Tylenol) during pregnancy and early childhood** | Acetaminophen during pregnancy is associated with hyperactivity, ADHD, and autism (the latter with a stronger association for men). Babies and young children exhibit an even greater association between acetaminophen and autism, likely due to lower detoxification capacities. Suggests women, babies and young children should limit acetaminophen intake. | [26, 105-107] |
| **Depression during pregnancy or use of antidepressant before and during pregnancy** | Depression during pregnancy is associated with an increased risk of having a child with autism, regardless of antidepressant use. However, there is also an association between maternal antidepressant use and the risk of autism in offspring that is particularly significant even before conception. | [108-110] |

| RISK FACTORS | COMMENTS | REFERENCE |
|---|---|---|
| **Use of other medication during pregnancy** | Thalidomide and valproic acid during pregnancy induces abnormal neuron development, which may be involved in the development of autism in the offspring | [111] |
| **Nutritional deficiency during pregnancy** | Maternal intake of folic acid and multivitamins before and during pregnancy is significantly associated with a decreased risk of autism in the offspring, particularly in high-risk families. Folic acid intake may mitigate the risk of pesticide exposure and autism. Lower uptake of essential elements like manganese and zinc is also observed in offspring with autism. Dysregulation of metal metabolism (including the zinc-copper cycles) are common in children with autism. Vitamin D deficiency during pregnancy is associated with an increased risk of autism, and is readily preventable with supplementation. | [14, 24, 112-119] |
| **Maternal and grandmother smoking during pregnancy** | Maternal smoking during pregnancy is associated with an increased risk of autism and ADHD in children in some studies, but not all. Grandmother smoking during pregnancy is associated with an elevated risk of autism in grandchildren. This may be due to exposure of the developing ovaries to cigarette toxicants, or to mutations in the mitochondrial DNA, which lead to an effect in the second generation. | [120, 121] |
| **Advanced parental age** | The risk of autism increases with both maternal and paternal age, independently, and this has implications for public health planning and the investigation of the etiology of autism. | [122-124] |
| **Environmental toxicants, endocrine-disrupting compounds, plastic derived chemicals, heavy metal, air pollutants, consumer products and pesticides** | Higher levels of some organochlorine compounds during pregnancy (PCBs, organochlorine pesticides) are associated with autism. Prenatal exposure to arsenic or manganese, and postnatal exposure to lead are associated with lower IQ and neurodevelopmental disorders. Children with autism may exhibit a higher uptake of the neurotoxin lead. Air pollution during pregnancy may be associated with an elevated risk of autism in children, and this may be an interaction between the air pollutants and offspring genes. Air pollutants of concern mentioned in autism studies include: particulate matter, heavy metals, styrene, chromium, PAHs and methylene chloride or other pollutants originating from highways. Some symptoms of autism (including emotional reactivity, aggressive behaviour, inattention symptoms, cognitive, motor or language disabilities) may be associated with plastic-derived chemicals such as bisphenols or phthalates (but not in all studies). Several endocrine disruptors affect thyroid signalling and may play a role in the development of autism. | [14, 16, 18, 36, 38, 114, 120, 125-128] |



# APPENDIX 2

History of pesticides and their epidemiological or mechanistic evidence linking them to autism.

## INSECTICIDES

**Organochlorines** – Originally used to control mosquito-borne malaria, organochlorines such as DDT were banned in the Unites States in 1972 [129]. Beside DDT, examples of organochlorines include endosulfan and dicofol [18]. Organochlorines can cross the intestine, the skin and the blood-brain barrier [130]. Being particularly persistent, they remain in circulation in the environment long after their application and continue to pose a risk to human health [131, 132]. However, when comparing the increasing trend of autism with the generally decreasing trend of organochlorine pesticide use would not make this family of pesticide a lone suspect to explain epidemiologic trends [22]. Nevertheless, there is an association between organochlorine exposure in the first trimester and autism for people living near agricultural fields [42]. Critical periods of vulnerability to organochlorine insecticide, which have been linked to autism, range from one month prior to conception to five months after conception and approximately two to eight months after birth [133]. While one study failed to find a linear dose-response association between organochlorine pesticide prenatal exposure and autism, further studies based on non-linear trends with decile analyses are warranted. A recent epidemiological study in Southern California quantifying trans-Nonachlor and p,p'-DDE in maternal mid-pregnancy serum samples reported biomarker based evidence associating maternal exposure to insecticides with autism among offspring [38]. Beside associations with autism, maternal exposure to DDT and DDE is associated

with premature birth and small gestational age status [40]. The DDT metabolite (DDE) has also been implicated in lower cognitive, verbal, memory and neurodevelopmental scores [134] and other neurodevelopmental deficits, such as decreased reflex response, psychomotor and mental effects, attention problems, hyperactivity disorder and pervasive developmental disorder, the former term for ASD [2]. One of the proposed mechanisms of action is through modulation of male hormonal signalling. DDE is a known androgen receptor binding inhibitor, androgen transcriptional activity inducer and androgen action modulator [135]. Lowered dehydroepiandrosterone (DHEA) levels have been observed in adult individuals with autism [136].

**Organophosphates** – Originally manufactured to replace the more toxic organochlorines, organophosphates unfortunately also pose a risk to human health [18]. They have short to moderate environmental persistence (half-life of 11 to 180 days in aerobic soils) but have been used in large volumes on farms and in homes. Examples of organophosphates include chlorpyrifos, dichlorvos and malathion [18]. Despite domestic-use ban in 2001 by the US EPA, they are still used in agriculture. Organophosphates kill pest insects by inhibiting acetylcholinesterase and by causing nerve damage [137].

A systematic literature review found an association between higher organophosphates exposure and altered neurodevelopment (in 26 out of 27 studies) [23]. This response was dose-dependent in all but 1 of the 12 studies that assessed organophosphorus exposure [23]. Longitudinal studies that assessed prenatal exposure to organophosphorous revealed cognitive deficits (working memory) at 7 years of age, behavioural (attention) deficits in toddlers and motor deficits (abnormal reflexes) in newborns [23]. At any moment in pregnancy, exposure to agriculturally used organophosphates is associated with elevated risks of autism [42]. In a 2019 study, risk of autism spectrum disorder was associated with prenatal exposure to three organophosphates, namely chlorpyrifos (OR = 1.13, 95% CI: 1.1–1.2), diazinon (OR = 1.1, 95% CI: 1.0–1.2) and malathion (OR = 1.1, 95% CI: 1.0–1.2) [41]. For autism spectrum disorder with comorbid intellectual disability, estimated odds ratios were higher (by about 30%) for prenatal exposure to chlorpyrifos (OR = 1.3, 95% CI: 1.0–1.6) and diazinon (OR = 1.41, 95% CI: 1.2–1.7) [41].

Symptoms of pervasive developmental disorder, the former term for ASD, were associated with higher organophosphates metabolites in the urine [138] or chlorpyrifos in umbilical cord blood plasma [139] in two different studies. Organophosphates have been shown to interfere with thyroid hormone, in animal models and in humans [140], and this may be a plausible biological mechanism for autism. Organophosphate may also induce other behavioural and cognitive deficits [141, 142] as well as differences in brain volume [143]. Organophosphate exposure has also been associated with loss of IQ points and intellectual disability, which has incurred costs ranging between Đ46.8 billion to Đ194 billion per year in Europe [35].

Chlorpyrifos is the most widely used organophosphate in the United States and in Europe [35]. It is also one of the top 5 most hazardous pesticides in Quebec both for the environment and for human health [144]. There is strong evidence that it is an endocrine-disrupting chemical, inhibiting acetyl-cholinesterase, but it can also induce neurodevelopmental toxicity through other mechanisms, such as interference with thyroid hormone signalling [35]. Higher concentrations of chlorpyrifos in the umbilical cord plasma has been associated with heightened risks of pervasive developmental disorders, the former term for ASD, in 3-year-old children [145]. Children with higher concentrations of chlorpyrifos have structural changes in the brain regions that are linked to attention, social cognition and receptive language processing [146]. Organophosphates can induce oxidative stress, mitochondrial dysfunction and cytotoxicity to neurons and liver cells [147, 148].

**Pyrethroids** – Pyrethrins are naturally derived insecticides from chrysanthemum flowers. Pyrethroids are their chemically synthesized relatives that are more persistent in the environment. Examples include permethrin and cyfluthrin. Just as organophosphates replaced organochlorines when the latter were found toxic and banned, pyrethroids heavily replaced organophosphates when these were in turn found toxic and banned. Once again, pyrethroids are not devoid of toxicity. They have been shown to influence neurodevelopment through a variety of mechanisms including interference with serotogenic systems, altered GABA function, oxidative stress, mitochondrial dysfunction, endocrine disruption and alteration in calcium signalling [14, 18]. In a 2019 study, the risk of autism was associated with prenatal exposure to the pyrethroid permethrin (OR = 1.10, 95% CI: 1.0, 1.2) [41]. For autism with comorbid intellectual disability, estimated odds ratios were higher (by about 30%) for prenatal exposure to permethrin (OR = 1.5, 95% CI: 1.2–1.8) [41].

Despite warnings against the use of pesticides during pregnancy, 88% of American pregnant women surveyed between 2000 and 2008 reported using pesticides at some point (mostly pyrethroids after the restrictions on organophosphates) [149]. Moreover, up to 75% of American children and adults, and up to 80% of teenagers, had urinary metabolites of pyrethroids [150]. Because pyrethroid metabolites are normally short-lived in the body, this high frequency of detection in the urine suggests that we are constantly exposed to pyrethroids in our daily living.

## HERBICIDES

**Glyphosate** – The link between glyphosate and autism is a topic of intense research and debate in the scientific literature. As proof, searching Google Scholar for the terms glyphosate and autism yields 1,300 results. But through those numerous references, only a few contained theories on the modes of action, many of which are considered speculative. But the authors warrant further scientific studies to clarify the potential role of exposure to glyphosate in triggering autism. First, correlations between the increase in use of glyphosate and the rise of autism have already been exposed. When comparing the rising trend of autism with the exposure trends to the top 10 toxic compounds encountered in the United States between 1980 to 2005, only three chemicals – including glyphosate – show a positive correlation (co-rising trends) [22]. Co-occurring rising trends have strikingly high correlations values in another study (R = 0.989 [53]). Of course, even a near-perfect correlation does not imply causation. But because glyphosate is the most widely sold pesticide on the planet, a strong correlation between the alarming rise of a developmental disorder and a toxic chemical cannot be simply dismissed without further studies [54]. In parallel with the ecological studies linking glyphosate and autism, other studies observed an excess of ADHD amongst children born to glyphosate appliers in South America [151] as well as glyphosate use and ADHD diagnosis in the United States [152]. In 2019, a new epidemiological study reported that autism was associated with prenatal exposure to glyphosate (OR = 1.2; 95% CI: 1.1–1.3). The risk for autism coupled to comorbid intellectual disability was even higher (about 30%) for prenatal exposure to glyphosate (OR = 1.33, 95% CI: 1.1–1.7) [41].

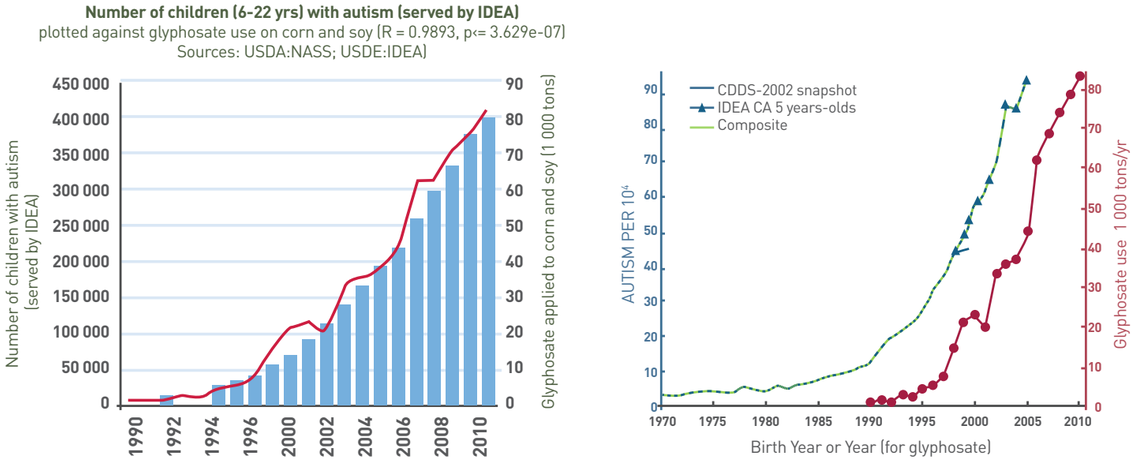
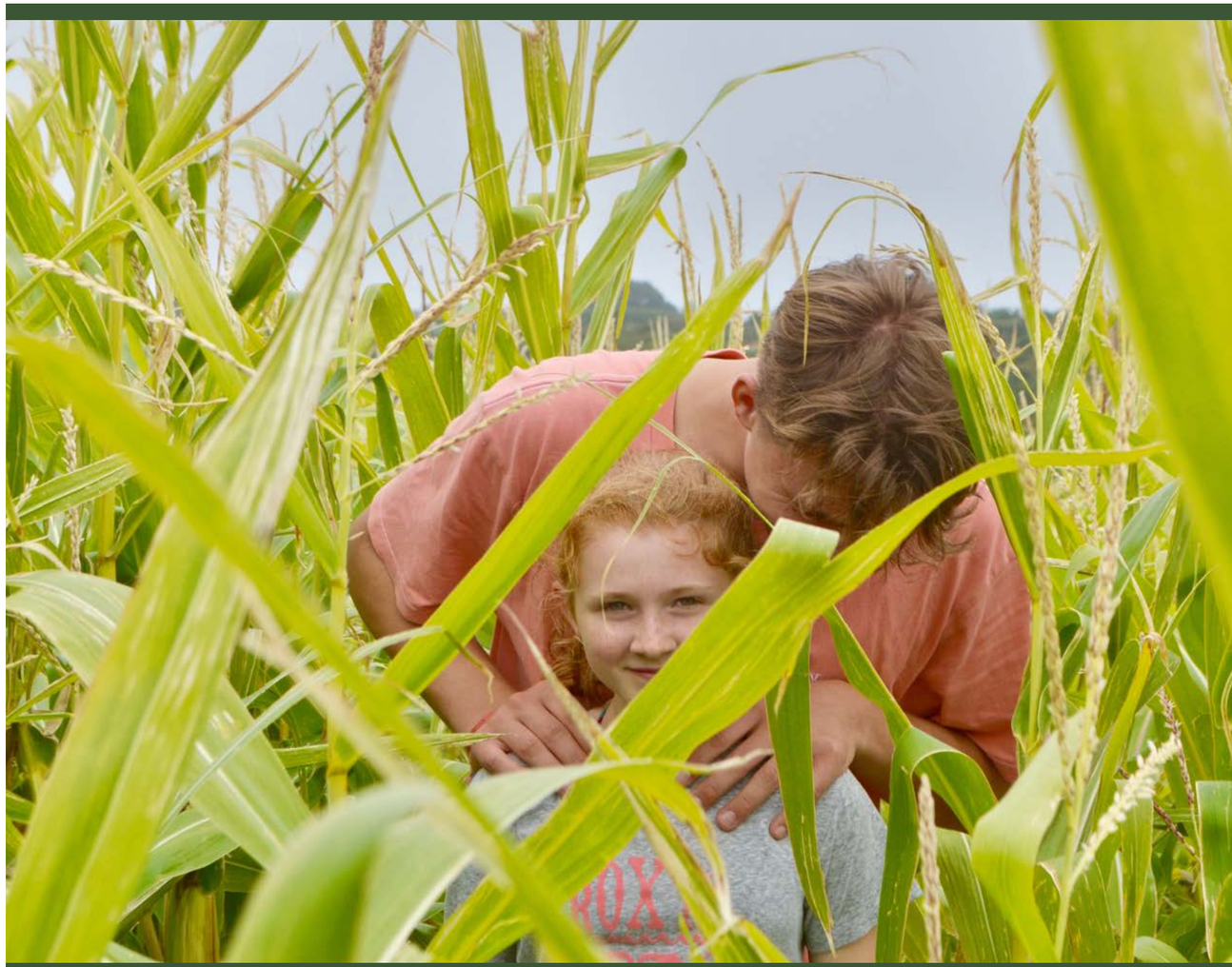



**FIGURE 4:** Increasing trends of autism and glyphosate use in the United States. Image sources: Left, using glyphosate applied to GMO corn and soy [53] and right, using all glyphosate applications [22].

Mechanistic pathways that could explain the observed correlation were proposed by the researchers Samsell and Seneff. One proposition involved glyphosate's mimicry of the amino acid glycine that could disrupt calcium inflow in immature neurons [55]. Another hypothesis is the manganese chelation capacity of glyphosate, which could lead to impaired mitochondrial function and impaired ability to detoxify glutamate, which are both linked to autism [56]. Finally, Beecham and colleagues proposed that by chelating metals (a known characteristic of glyphosate), this active ingredient could hamper the functioning of a manganese-dependent enzyme leading to a reduction in thyroid-stimulating hormone (TSH), a condition itself correlated with increased autism risk in mid-pregnancy mothers [54]. The authors of this report warrant a focused research effort to clarify this. Indeed, we need further research on the potential links between glyphosate and autism. Some have argued that some of the mechanisms proposed by the team of Samsell and Seneff are speculative [153]. Even severe critiques of these theories nevertheless advocate for better scientific studies to enlighten this debate [154] and that studies supporting glyphosate's safety are generally funded by the agrochemical industry, whereas those suggesting toxicity below the set safety limit generally come from independent researchers [153]. Another important theory potentially implying glyphosate – a patented antibiotic – and autism involves action on the gut microbiota [155]. Indeed, the potential role of antibiotics and alteration of the gut microbiota and its putative role in autism is increasing in popularity in the scientific community [156]. At the same time, the effect of glyphosate on gut bacteria is not required in registration testing and symptoms in test animals, including diarrhea and loose stools, and are often dismissed as non-specific [157]. At the very least, this theory too deserves further study.



**Ammonium Glufosinate** – As weed resistance to glyphosate is increasing, a rising alternative herbicide is a close chemical relative called ammonium glufosinate. As for most pesticides, studies on potential adverse effects related to developmental neurotoxicity are lacking. Recently, a study on mice revealed that in utero exposure led to offspring that displayed symptoms strikingly similar to animal models of autistic spectrum disorders, including early reflex development, pup communication, affiliative behaviours, and preference for social olfactory cues [49]. These behavioural traits were also concomitant to biological changes implying impaired neurogenesis and neuroblast migration through cytoskeleton destabilization [50]. This is the first evidence of the link between early life exposure to the herbicide and molecular and cellular consequences and the onset of ASD-like phenotype later in life. Neural stem cells of the subventricular zone of the developing brain can be affected both by ammonium glufosinate and its main metabolite 4-methylphosphinico-2-oxobutanoic acid through different modes of action [57]. Finally, ammonium glufosinate also leads to neurotoxicity and altered behaviour in reptiles, and this effect is exacerbated in males compared to females [158].



# APPENDIX 3

## Pesticide families associated with autism in various epidemiological studies

This table was reproduced from a systematic literature review [14]

| Environmental Factor and Study Reference, Location, and Name | Study Design | Exposure Assessment | Results | State of the Evidence |
|---|---|---|---|---|
| **PESTICIDES** | | | | |
| **ORGANOPHOSPHATES (OP)** | | | | |
| Rauh et al., 2006 New York, USA | Prospective cohort (n=228; ←5% PDD cases) | Measures of chlorpyrifos in plasma (cord or maternal) | Highest chlorpyrifos exposure group had greater risk for PDD as defined by scores on Child Behavior Checklist (CBCL) | Four studies using different methods consistently show elevated risk. Exposure measurements or other information all pertain to the prenatal period. Outcomes not based on clinical assessment in majority of studies. Studies suggest potential association between an organophosphate pesticide and ASD or related symptoms. However, use of one or two isolated measurements may not provide valid surrogates for overall prenatal or infant exposures. Further research with confirmation of diagnoses using gold standard protocols and better measures of individual-level exposures over time is needed. |
| Eskenazi et al., 2007 California, USA (CHAMACOS) | Prospective cohort (n=355; 51 PDD cases) | Prenatal & child OP (organophosphate) urinary metabolite levels | Prenatal and postnatal dialkylphosphate (DAP) metabolites associated with more than two-fold higher risk for PDD as defined by scores on Child Behavior Checklist | |
| Roberts E., et al., 2007 California, USA | Case-control (n=465 cases and 6,975 matched controls) | Proximity to agricultural applications of organophosphates | ASD community diagnosis modestly associated with organophosphate applications within 250m, during gestation | |
| Shelton J.F., et al., 2014 California, USA (CHARGE) | Case-control (486 ASD cases; 316 typically developing population controls) | Proximity to agricultural applications of organophosphates | Clinically confirmed ASD diagnosis associated with organophosphate applications within 1.5 km during pregnancy, particularly chlorpyrifos during 2nd trimester | |
| **OTHER PESTICIDES** | | | | |
| Roberts E., et al., 2007 California, USA | Case-control (n=465 cases and 6,975 matched controls) | Proximity to agricultural applications of organo-chlorine insecticides, or pyrethroid insecticides | ASD community diagnosis strongly associated with residential proximity to organochlorine applications during 1st trimester, and moderately for the pyrethroid, bifenthrin, during the overall gestation | Analyses from one report suggesting a strong association of ASD with organochlorines and a moderate one with a pyrethroid require confirmation in independent samples, preferably with gold standard diagnoses. Results on imidacloprid potentially related to differences in reporting accuracy between cases and controls. |
| Keil et al., 2014 California, USA (CHARGE) | Population-based case-control (n=407 ASD, and 262 typically developing controls) | Maternal report of common flea or tick treatment for pets (imidacloprid) | Using Bayesian methods, no overall association. Higher risk in those with frequent use. Sensitivity analyses to address misclassification yielded inconclusive results | |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