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Sex-Specific Neurotoxic Effects of Organophosphate Pesticides Across the Life Course

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Abstract
Purpose of Review This review discusses the sex-specific effects of exposure to various organophosphate (OP) pesticides throughout the life course and potential reasons for the differential vulnerabilities observed across sexes.
Recent Findings Sex is a crucial factor in the response to toxicants, yet the sex-specific effects of OP exposure, particularly in juveniles and adults, remain unresolved. This is largely due to study design and inconsistencies in exposure and outcome assessments.
Summary Exposure to OPs results in multiple adverse outcomes influenced by many factors including sex. Reported sex-specific effects suggest that males are more susceptible to OPs, which reflects the sex-dependent prevalence of various neurodevelopmental and neurodegenerative disorders such as autism and amyotrophic lateral sclerosis (ALS), in which males are at greater risk. Thus, this review proposes that the biological sex-specific effects elicited by OP exposure may in part underlie the dimorphic susceptibilities observed in neurological disorders. Understanding the immediate and long-term effects of OP exposure across sexes will be critical in advancing our understanding of OP-induced neurotoxicity and disease.

Keywords Sex · Sexual dimorphism · Organophosphate · Pesticides · Neurodevelopment · Neurodegenerative diseases

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Introduction

The term "sexual dimorphism" refers to the expression of differences at all levels of biological organization (e.g., anatomy and function) beyond the sex organs between females and males of the same species. Until recently, sexual dimorphisms have been largely disregarded in the study design of most investigations of physiological or pathological processes in humans, cell cultures, and model organisms across all the disciplines of biology and medicine. This is because it was thought that effects of biological importance would be seen independently of sex. Yet, today it is impossible to ignore the differences between females and males in regard to vulnerability to diseases, exposure to exogenous agents, and response to therapeutic interventions, as highlighted in a 2001 Institute of Medicine (IOM) report [1]. This report has been instrumental in stressing the necessity for research investigations from mechanistic to epidemiological studies to examine both sexes concurrently. Other policies, including the US National Institutes of Health requiring sex to be considered as a variable in research studies, have followed suit after the release of this report.

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Thus, an outstanding question in neurotoxicology is how exogenous agents modify brain function and subsequent behavior as a function of sex [2]. In this review, we summarize and discuss the current knowledge regarding sex-specific neurotoxic effects of organophosphate (OP) pesticides as it exemplifies how our knowledge on sex differences is still sparse. By research design, the question has been eluded for years; most epidemiological studies adjust for sex in the analyses, and in vitro studies use cells with undetermined sex origin or produced from the two sexes mixed, whereas in vivo animal studies have provided compelling evidence of significant sex-dependent effects. Of note, we acknowledge that this critique of the limitations of previous studies is also a self-critique that we hope will invite other investigators—like us—to rethink their research strategy to improve the field.

Exposure to OPs represents an especially insightful example for discussion as these neurotoxicants have differential outcomes particularly dependent on the timing of their exposure throughout the life course. There are indications that their impact may range from the sexual differentiation of the brain itself [3] to the development of certain adult-onset neurodegenerative diseases that display sex differences in their patterns [4–9]. Therefore, in addition to summarizing existing evidence of sex-dependent neurotoxic effects of commonly studied OP pesticides when exposed at different stages of development, this review also discusses the possible relationship between OP exposure and late-onset neurodegenerative diseases that show similar sex-dependent patterns.

Organophosphate Pesticides: Ubiquitous Neurotoxins

OPs are a class of widely used agricultural pesticides that were also developed for use as warfare nerve agents and other chemicals with numerous applications in clinical, household, and industrial settings; for review, see [10]. OPs account for one third of the total pesticide usage annually in the USA [11]. Among OPs, chlorpyrifos (CPF) is still one of the most widespread pesticides; in the USA, more than eight million pounds of CPF are used each year for agricultural purposes, although the Environmental Protection Agency (EPA) has banned its indoor residential use since 2000 [12]. Since agricultural use of CPF persists, this compound has frequently been detected in air, food, and water in the USA. CPF-specific metabolites detected in human urine samples also suggest that exposure remains ubiquitous [13]. OPs, such as CPF, have a very short elimination half-life (~ 27 h [14]), and thus, metabolites reflect ongoing or recent exposure. For all these reasons, CPF has been the most studied OP in both epidemiological and experimental studies and is considered a compound representative of the entire OP family that also includes diazinon, dichlorvos, diisopropylfluorophosphate, malathion, methyl parathion, and triorthocresyl phosphate. (For information on CPF, see [15]; for general information on OPs, see [16].)

Some OPs are well known for their potentially deadly neurotoxicity in cases of accidental, criminal, or suicidal exposure to high doses, a concerning problem mainly in developing countries where their use is poorly regulated [17]. This acute neurotoxicity is well characterized and is mediated by inhibition of acetylcholinesterase (AChE), the enzyme that inactivates the neurotransmitter acetylcholine after its release at the cholinergic synapse (see [18] for review). This results in massive accumulation of acetylcholine at the synaptic cleft, excessive stimulation, and subsequently widespread impairment of all the physiological functions controlled by the cholinergic system (e.g., blood pressure, cardiac rhythm, and muscle contraction). Ultimately, the cholinergic crisis can lead to death by respiratory failure.

While acute toxicity is still relevant, especially in agricultural workers, in this review we focus our attention on chronic, low-dose OP exposure that does not elicit overt toxicity because this represents the exposure of the majority of the world's population. Neurotoxicity resulting from low-dose exposure is thought to occur mainly through non-cholinergic processes that remain unclear, yet proposed mechanisms include cytotoxicity, abnormal neuronal cytoarchitecture, aberrant energy homeostasis and neurotransmission, neuroinflammation, and blood-brain barrier impairment (reviewed in [19•]). Such low-dose exposure can begin as early as gestation; OPs can pass through the placenta and amniotic fluid to the fetus [20]. Moreover, a fetal or child brain is particularly vulnerable to the effects of OPs due to the rapid morphological development occurring; OP toxicity can disrupt many processes (e.g., cell division, migration, differentiation, and establishment of synapses and networks) and therefore alter the developmental trajectory of the nervous system. Brain malformations in this critical period can thus result in consequences that persist long after exposure ends [21]. Also, compared to adults, fetuses and children have lower levels of detoxifying enzymes (e.g., paraoxonase 1), which can deactivate OPs [22]. The smaller body weights and faster metabolisms of children cause rapid bioactivation of some non-toxic parent OPs into toxic metabolites (e.g., CPF into CPF-oxon); furthermore, behaviors such as increased hand-to-mouth activity account for the overall higher OP exposures measured in children than in adults, putting developing children at greater risk for adverse outcomes associated with higher exposure [23].

While developmental neurotoxicity has received the most attention, adolescent and adulthood exposure is still a cause for concern as exemplified by the different adverse effects and diseases that are associated with occupational exposures [24–26] (for reviews on long-term exposure and neurological disease, see [27, 28]). On the other hand, OP exposure in a mature brain does not cause as dramatic a disruption as in a developing brain because neural networks are already established and post-mitotic cells likely evolved to be more

resilient to toxic insults—a defense potentially reversed by aging and disease.

Establishment of Sexual Differentiation in the Brain

The processes involved in programming the sexual differentiation of female and male brains have been reviewed elsewhere (see [2, 29]). In brief, the long-standing dogma has been that a male's brain undergoes active hormonal masculinization while the female brain is the default state. We now know that sexual differentiation requires more than sex hormones. For instance, multiple sex differences in the mammalian brain and associated behaviors can be attributed to chromosome complement (i.e., XX vs. XY) rather than gonadal phenotype [30]; for review see [31]. In addition, neuroimmunity and neuroepigenetics have recently been identified as two mediators instrumental in the establishment of sexually dimorphic brains, as reviewed in [29]. The combination of these mechanisms leads to numerous differences between female and male brains such as differences in neurochemical phenotype, synapses, cell genesis, and brain volume [29]. Thus, the multiple structural levels of sexual dimorphisms in the brain result in a myriad of differential targets for neurotoxicants across sexes, providing biological plausibility for various mechanisms of sex-dependent effects of neurotoxic compounds such as OPs.

Deciphering when the central nervous system (CNS) is most vulnerable to neurotoxic exposures is of critical interest to neurotoxicologists. Yet, this matter is complex given that the window of vulnerability depends not only on the nature of the neurotoxicant itself but also on its capacity to enter the CNS across the life course (e.g., Does the neurotoxicant cross the placental barrier? Is it detected in breast milk? Can it only permeate the immature blood-brain barrier, or rather is it more permeable to the leaky, aged, or diseased blood-brain barrier, or does it penetrate indiscriminately?). OPs can penetrate the CNS throughout the life course, although to varying degrees; when applicable, we will discuss how sex differences in OP metabolism can also be a source of variation in internal exposure and possible associated negative outcomes. Another important factor to consider is the outcome studied as there may be differential windows of vulnerability for different outcomes. As previously mentioned, fetal and child brains are particularly vulnerable at critical periods of growth and development, when major functional organization is established [32]. Yet in the adult brain, the maturation of certain structures may render them suddenly vulnerable to neurotoxic processes from which they were protected during development due to a change in neurochemistry, connectivity, or metabolism. Also, the aged brain may be at the highest risk for adverse effects of OP exposure due to decreased antioxidant and immune defense and a leaky blood-brain barrier. For these reasons, we have organized the structure of this review based on the timing (or predicted window) of OP exposure.

Methods

Inclusion/Exclusion Criteria

Throughout this review, we systematically included all the epidemiological studies that considered sex-dependent effects (whether they found positive or negative results) in which OP exposure was ascertained by individual biomarkers of internal exposure (e.g., most commonly, measurement of dialkyl phosphates [DAPs], a group of OP metabolites) as this provides the highest degree of confidence in our comparison of existing human and animal data. Most studies in which OP exposure was assessed by self-report or other non-biological metrics were excluded, with the exception of few particularly pertinent to the discussion of other studies with ascertained individual exposure. For experimental studies, we decided to include only in vivo and in vitro (for which we found only one study) experiments performed in mammals/mammalian tissue due to the greater similitude to human neurodevelopment. We did not find any pertinent studies performed in orders of mammals besides rodents. We would also like to disclose that although several of the studies we will discuss indiscriminately use both the terms "gender" or "sex" to refer to sexual dimorphisms, in this review we will only use the word "sex" to refer to biological classification, as all of the references cited here classify gender based on sex at birth rather than self-perceived gender.

Literature Searches

PubMed searches were performed between April and June 2017, using the following terms:

> "organophosphate"[Title/Abstract] OR "organophosphorus"[Title/Abstract] OR "organophosphorus"[Title/Abstract] OR individual OP names e.g. "chlorpyrifos"[Title/Abstract] OR "diazinon"[Title/Abstract] OR "dichlorvos"[Title/Abstract] OR "diisopropylfluorophosphate"[Title/Abstract] OR "malathion"[Title/Abstract] OR "methylparathion"[Title/Abstract] OR "parathion"[Title/Abstract] OR "triorthocresyl phosphate"[Title/Abstract] **AND** "sex-specific"[All Fields] OR "sex-dependent"[All Fields] OR "sex-selective"[All Fields] OR "gender-specific"[All Fields] OR "gender-dependent"[All Fields] OR "gender-selective"[All Fields] OR "sexual dimorphism"[All Fields] OR "sexually dimorphic"[All Fields] OR "males"[Title/Abstract] OR "females"[Title/Abstract] OR "boys"[Title/Abstract] OR "girls"[Title/Abstract] OR "men"[Title/Abstract] OR "women"[Title/Abstract] **AND** "brain"[Title/Abstract] OR "neurodevelopment"[Title/Abstract] OR "neurodevelopmental"[Title/Abstract] OR "nervous

system"[Title/Abstract] OR "neurons"[Title/Abstract] OR "glia"[Title/Abstract] OR "neurological"[Title/Abstract] OR "cognitive"[Title/Abstract] OR "mental"[Title/Abstract] OR "neurobehavioral"[Title/Abstract].

Sex-Specific Effects of OP Exposure During Prenatal Brain Development

Epidemiological Evidence

As previously mentioned, exposure to OPs often begins prenatally [20]. Prenatal OP exposure has been associated with a variety of adverse effects on neurobehavioral development in infants and children such as changes in body length and abnormal neonatal reflexes [33, 34], poorer mental development [35, 36], cognitive impairments [37–39], attention problems [40, 41], and other effects reviewed elsewhere [42•, 43, 44]. Yet, limited epidemiological evidence exists in support of sexually dimorphic responses to developmental OP exposure because most studies adjust for sex (e.g., [38, 40, 45]) rather than look for the possibility of different associations by sex. Simple adjustment has been criticized for essentially stripping sex differences from the analysis [46]. Many other studies barely mention sex at all except as a demographic variable, e.g., [47, 48]. Still, as we will describe in the following paragraphs, sex-specific responses related to attention, working memory, social behavior, brain morphology, and motor skills and development have been reported following prenatal OP exposure (summarized in Appendix Table 1).

The association between maternal urinary OP metabolite levels (DAPs) during the second trimester of pregnancy and attention problems and attention deficit/hyperactivity disorder (ADHD) scores measured in 3.5- and 5-year-old children (by maternal report, psychometrician observation, and neuropsychological testing) was significant in boys but not in girls [40]. Interestingly, this result reflects the pattern of ADHD diagnosis, with males being more affected [49]. A more recent study also found trends towards an increased ADHD index in boys ages 6–11 years exposed to CPF or CPF-methyl prenatally, with exposure measured by presence of the specific metabolite 3,5,6-trichloro-2-pyridinol (TCP_Y) in maternal urine during the third trimester of pregnancy [50]. A possible explanation for the borderline significance in this more recent study as compared to [40] could be the restriction here of the association to only one OP and its methyl derivate as compared to DAP metabolite levels which were proposed to cover more than 80% of all the local pesticides in [40]. It should be noted that although the exposure assessment in these studies occurred in different trimesters, levels of OP metabolites are not expected to change significantly during pregnancy. Indeed, repeated measures of a subset of mothers in [50] found no significant differences in geometric mean TCP_Y concentrations across trimesters. In contrast, a previous study did not find a similar sex-dependent association between early OP exposure and ADHD score in childhood [35]. However, unlike [40, 50], this study [35] assessed very young children at 6, 12, and 24 months of age, when attention deficits may not have been detected as this type of executive control is still highly immature in young brains [32].

Sex-specific effects have also been reported for working memory, with 7-year-old boys prenatally exposed to CPF experiencing a greater working memory deficit than girls, as assessed by the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV), and controlled by direct measure of CPF in umbilical cord blood at birth [51]. However, the sex-specific effect reported in this study also did not reach statistical significance. Possible explanations for the borderline significance are again the restriction of OP exposure assessment to the measure of CPF only and also the difficulty to capture direct CPF levels in a single sample because, as previously mentioned, CPF metabolizes very quickly [14] (e.g., only 60% of the samples were above the detection limit). A clear strength of the use of cord blood is that it more directly reflects child exposure than maternal biomarkers do. Measurement of DAPs, which are much more stable, is probably a better strategy to improve power for such studies. However, DAPs have been criticized due to their potential formation in the environment (e.g., on agricultural products) rather than by direct metabolism, and thus their measure may not be solely reflective of human exposure to the parent OPs.

Social skill deficits and their multifaceted forms—like pervasive development disorders, including autism—impact males more than females [52]. Therefore, studies have investigated the relationship between early-life OP exposure (assessed by maternal urinary metabolite concentrations), sex, and adverse social outcomes (assessed by the caregiver using the Social Responsiveness Scale, SRS). Studies have not found an association between DAPs as a whole and social functioning when assessed at ages 7–9 [53•, 54], although there may be an association between increasing levels of diethyl phosphates (a DAP that can be produced by CPF metabolism) and adverse SRS scores among boys [53•].

An innovative study using magnetic resonance imaging detected significant sex-by-exposure interactions in several cortical regions in children prenatally exposed to CPF [3]. The investigators observed disruptions of expected sex differences in both female and male brains in regions involved in "cognition, behavior, language, reward, emotion, and inhibitor control," suggesting impairment in the standard progression of sexual differentiation. Importantly, this study also detected an inverse dose-response relationship between prenatal CPF exposure (directly measured in umbilical cord blood at birth) and cortical thickness, weakening the likelihood of

alternative explanations being responsible for the observed results. Another study examining tremor in the same cohort found that among adolescents ages 11–14 prenatally exposed to CPF, boys were significantly more likely to demonstrate clinically meaningful tremor than girls, as assessed by a neurologist blinded to exposure status [55].

While the majority of studies suggest that males are more susceptible to the adverse effects of developmental OP exposure than females, recent evidence has suggested that for certain outcomes such as motor function [56] and neonate neurobehavioral development scores [57], female neonates may be more negatively impacted. This has similarly been demonstrated in rats, where late gestational exposure to CPF selectively impacted locomotor activity of females, detected as slower habituation in a locomotor assessment [21]. However, both of these epidemiological studies were conducted in China, where the mothers had geometric mean urinary DAP levels significantly higher than mothers in Western countries [57]. Thus, males may be more susceptible to the effects of moderate doses of OP whereas effects in females manifest only at much higher doses.

Experimental Evidence

In Vitro Evidence

In the search for in vitro studies using brain cell cultures and mentioning sex-specific effects of OP exposure or any other types of neurotoxicants, and even when the search is extended to include any type of neurologically related outcome, the immediate realization is that we are facing more than a knowledge gap: there is essentially no knowledge pertaining to sexual dimorphisms in studies conducted in cell culture. As emphasized in the previously mentioned IOM report, scientists tend to forget that "every cell has a sex" [1], not just by virtue of its chromosomes but also its environmental influences before it is isolated for experimental purposes. We are guilty ourselves of contributing to this general incuriosity by developing "unaware" sex (e.g., stem cell lines produced from embryos of unknown sex) or "nullified" sex (e.g., primary brain cells produced from animals of the two sexes pooled) in vitro models of the neurodegenerative disorder amyotrophic lateral sclerosis (ALS) [58, 59], which some evidence suggests is associated with OP exposure and has a higher incidence in males than in females, as we recently reviewed in [60]. Yet beyond the cells' origin, other masculinization or feminization factors should be taken into consideration, such as the presence of hormones in the culture medium [2]. The future of in vitro investigations of sexual dimorphisms will probably require manufacturers of culture sera to distinguish sera produced from male and female animals. In the context of this review, to the best of our knowledge only the in vitro study by Astiz and colleagues [61••] has paid close attention to the influence of sex. They found that the OP dimethoate selectively alters the production of inflammatory molecules, reactive oxygen species, steroidogenic proteins, and estrogen receptors in male cortical astrocytes, which could explain dimethoate's selective deleterious effects in male rats in vivo [62, 63].

Animal Prenatal Exposure: Mid-Gestation

In stark contrast with epidemiological and in vitro studies, sex-specific effects of gestational or early developmental OP exposure are commonly reported in rodent studies (see [42•] for a thorough review on developmental neurotoxicity of insecticides to date). While CPF is the most studied OP, sex-dependent effects in rodents have also been reported after exposure to other OPs including diazinon (DZ), dichlorvos, malathion, methyl parathion, and parathion (see Appendix Table 2 for a summary of sex-dependent effects in these less commonly studied OPs, as sex-dependent effects of CPF have been reviewed elsewhere [42•, 64]). In general, most of the studies we will discuss employ the following dosing regimen: administration via subcutaneous injection to the dam or neonate over a short period (usually 4 days), where the dose is below the threshold for overt cholinergic toxicity. The nature and magnitude of the neurotoxicity and whether sex-dependent effects are produced depend critically on the period of exposure [65–68], the particular organophosphate [69–71], and the species and genetic background of the animal and the nature of the behavior being tested [72, 73]. For example, short-term exposure to CPF mid-gestation (rodent gestation: 21 days; mid-gestation: gestational days 9.5–11), when the neural tube forms and neurogenesis is heightened in the developing rodent [74], did not produce sex-dependent effects in locomotor activity, prepulse inhibition, or memory [75]. However, females display locomotor and memory impairments when the exposure window for CPF is shifted to late gestation [21]. In humans, neural tube formation occurs comparatively earlier (in weeks 3–4 of gestation) and mid-gestation migration and maturation are already occurring in many structures while neurogenesis is still at play in others. Similar critical periods for toxicity and sex-dependent effects are seen for serotonin (5HT) synaptic function; e.g., while there is no effect on 5HT turnover in adult rats when CPF is given during mid-gestation (gestational days 9–12), there is a preferential effect in males when CPF is given in late gestation (gestational days 17–20) [66, 67]. OP exposure early in gestation affects both sexes equally likely because sexual differentiation of the brain occurs later in development [76].

Animal Prenatal Exposure: Late Gestation

CNS development and maturation proceed in roughly a caudal to rostral direction. Accordingly, during late gestation in rodents and humans alike, some structures have completed

neurogenesis and migration and are in the beginning phases of maturation (e.g., synaptogenesis) that will be completed postnatally. However, unlike in humans where neurogenesis continues for years, neurogenesis in rodents is almost entirely completed at birth [74]. Sex-specific behavioral effects resulting from late gestational exposure to CPF have been observed in locomotor activity [21], spatial learning and memory [69], social investigation [77], and affective states [78, 79], and in classically hormone-mediated sexually dimorphic behaviors such as aggression in males and maternal behaviors in females [80, 81], reviewed in [64]. Late gestational OP exposure alters the developmental trajectory of a variety of neurotransmitter systems (including serotoninergic, as previously mentioned, and catecholaminergic systems) in a sex-specific manner [65, 82–85], supporting the view that chronic low-dose OP exposure impacts a variety of neurotoxic targets different from the cholinergic system. However, interestingly the effects of OPs on non-classical neurotransmission systems, such as the one of the gaseous signaling molecule nitric oxide, are sex-independent [70, 86].

A recent study concluded that early (gestational day 15 to postnatal day 14) CPF exposure resulted in sex- and region-specific changes in estrogen receptor beta (*ERβ*) with concomitant sex-dependent behavioral alterations [87•]. It is noteworthy to point out that the exposure route in this study is very similar to the exposure in developing humans, for mothers and neonates were fed CPF through the diet, and exposure lasted from gestation until the end of lactation. Thus, sex-specific effects of developmental CPF (and perhaps other OP) exposure could be a result of its effects on *ERβ*, which also plays a role in the sexual differentiation of the brain.

Animal Postnatal Exposure

Early postnatal exposure in the pre-weaning period in rodents is equivalent to the third trimester in human gestational development [74], a period marked by synaptogenesis, neurotransmitter system alterations, axonal growth, and myelination. Various sex-dependent effects have been observed when OPs are administered to rats during this window critical to CNS maturation [65, 83, 88, 89]. Notably, all these studies that focused on the adverse effects of CPF exposure during postnatal days (PND) 1–4 observed a disruption of the normal expected sex differences in behavior and brain morphology, suggesting an impairment of brain sexual differentiation as has also been demonstrated in humans in response to CPF [3]. A comparable exposure paradigm administering DZ resulted in feminization of the typical male response in prepulse inhibition at 4–5 weeks, whereas other measures including locomotor activity and working memory were affected in both sexes equally [90]. The same DZ exposure resulted in sex differences in the elevated plus maze and novelty-suppressed feeding assay that persisted to adulthood, also preferentially affecting males [91]. However, a comparable exposure paradigm with CPF in outbred Swiss-derived (CD1) mice did not result in marked sex differences (as observed in rats) in cholinergic system-regulated behavioral end points as well as ultrasound emissions, orientation to home nest material in neonates, social/affiliative behaviors, and novelty-seeking behavior [80]. This is indication that species and possibly strain differences can alter the response to OP pesticides (i.e., a mouse strain other than CD1 may have yielded the same sex-dependent effects as observed in rats [90, 91]). Another important consideration is that in [80], the CD1 mice were from an outbred colony, i.e., with increased genetic variation in progenies, which could better emulate the genetic variability inherent to humans but also mask important effects that could be specific to certain genotypes found in different human populations. Therefore, each model system needs to be thoroughly considered for its similitudes and divergences with humans (e.g., metabolism, genetics, and developmental window equivalence) before extrapolation of the results.

In regards to the serotonergic system, PND1–4 exposure to DZ resulted in a significant decrease in 5HT1A receptors in males at the lower dose administered, whereas females showed an opposite trend [92]. Sex differences in neurochemical evaluations were also reported in response to early postnatal parathion exposure [71]. However, these results are in "stark contrast" to previous findings with CPF [66]. In general, the effects of parathion and DZ are similar in that they are smaller in magnitude and less persistent than CPF effects. This demonstrates that the serotonergic system is likely a susceptible target of OP toxicity. However, different OP compounds can elicit a range of effects; deciphering the molecular basis of this intriguing observation should provide important insights into the differential neurotoxic potential of OPs.

Lastly, a 2010 study that assessed the effects of early postnatal parathion exposure in rats is noteworthy because it is one of the only studies to examine whether the effects of exposure persist not just through adulthood (PND100) but also into early senescence (14–20 months of age) [93]. Interestingly, males showed greater deficiencies in working memory, assessed by the radial arm maze. Markers of 5HT and acetylcholine synaptic function measured in corresponding neurochemical assays provide a mechanistic clue to this observation. These results suggest that impairments may begin to emerge when the CNS declines with aging and that exposure history is indeed relevant throughout the entire lifespan. The fact that such brief, low-dose exposure early in development can have effects that persist into senescence is critical because these doses are comparable to human exposures. Further experimental investigation in rodents is needed as well as longitudinal analyses of early-life exposure in epidemiological studies.

Few studies have examined the effect of late postnatal OP exposure (what we are considering after the second postnatal week, PND14). Jett and colleagues [94] found no sex

differences in the Morris water maze following administration of CPF, while sex differences have been observed following exposure of diisopropylfluorophosphate (PND14–20), with females selectively displaying learning and memory impairments [95]. This further demonstrates that specific OPs can have unique effects and that they may have particular windows in which they exert neurotoxicity.

Most animal studies in the literature administer the OP for a brief dosing regimen (4–10-day duration); however, humans are likely exposed not only during gestation but chronically, throughout weaning and childhood as well, as OPs have been detected in breast milk [96] and measured in children. One experimental study assessed the effects of a more chronic dosing schedule (PND1–21) of either CPF or methyl parathion and found that both OPs impacted working memory of males while females remained unaffected [68]. Furthermore, methyl parathion caused an impairment at a lower dose than CPF. Deficiencies in reference memory also preferentially impacted males (at the highest dose of CPF only and all doses of methyl parathion). These findings are significant because this represents a dosing regimen more relevant to human exposures (chronic, administered orally via gavage) and demonstrates that while working memory deficits preferentially impact males, they have varying sensitivity to different OPs.

Sex-Specific Effects of Childhood OP Exposure

Epidemiological Evidence

Understanding the effects of OP exposures among children (3.5–18 years old) is critical because as described in the "Introduction" section, they have higher exposures than the rest of the population [13] for various behavioral and physiological reasons. Also, adolescents in developing countries frequently work as pesticide applicators, increasing their risk for acute exposure to high levels of OPs and adverse effects due to chronic exposures [24, 97, 98]. Additionally, adolescent brains undergo rapid development due to hormonal and corresponding physiological changes associated with puberty, rendering them even more sensitive.

Sex-dependent effects of childhood OP exposure have been reported in assessments of neuropsychological development including cognition, verbal comprehension, attention, memory, and coordination (Appendix Table 1). For example, a recent study innovatively used a geographic information system (in addition to urinary DAP metabolites) to assess effects of cumulative OP exposure on neuropsychological development, measured by the WISC-IV, in children ages 6–11 years [99]. They discovered that higher postnatal OP exposure was associated with decreased intelligence quotient (IQ) and verbal comprehension, with the effects more pronounced in boys than in girls. This study is significant because many other studies in the literature that do not detect sex-specific effects are cross-sectional in nature and thus neglect the contribution of cumulative exposures during gestation and after birth.

Another study found that lower erythrocytic AChE activity in children ages 4–9 was associated with poorer neurodevelopment in boys, but not girls, particularly in attention, inhibition, and memory [100]. Neurodevelopment was measured using the NEPSY-II, an assessment that measures various developmental and neuropsychological domains. It is worth mentioning that functional decrease in AChE activity has the merit to be a biomarker of effect rather than exposure, but may be sensitive only to higher than average exposures (e.g., in children raised in agricultural areas with heavy OP use).

Unfortunately, all other studies that reported sex differences in response to childhood/adolescent OP exposure have only employed non-biological exposure measurements, such as classifying the "exposed" group as those residing in an agricultural region. Researchers detected sex-specific effects in motor speed and response latency in males ages 2–6 years of age [101], and in the computerized Behavioral Assessment and Research System (BARS) comparing adolescent (ages 10–18) farm workers with urban residents [102]. Interestingly, the strongest effect was seen in the youngest participants (ages 10–11). This supports the notion that the adverse effects of exposure are stronger when exposure happens earlier in development. Or, it may suggest that some neurodevelopmental effects of OP exposure (e.g., deficits in complex function and coordination) are transient. This further highlights the need for longitudinal assessment. Yet again, the lack of individual exposure assessment limits the interpretation of these results.

There are still many gaps in the literature. Studies that have conducted repeated longitudinal measurements assessed male adolescents only and thus cannot assess sexually dimorphic responses, e.g., [24, 98, 103]. In addition, still very little is known about the risks of average- or low-dose exposure, which is relevant to most of the US adolescent population, as most studies have focused on adolescent populations with high levels of exposure, such as those living in agricultural areas or working as pesticide applicators (in large part because these adolescents are at the greatest risk) [104]. Finally, while there are many animal studies assessing developmental neurotoxicity of OPs, experimental studies have not explored the effects of juvenile exposure, likely because in rodents this is difficult to study due to their rapid development during this period [105].

Sex-Specific Effects of OP Exposure in Adulthood

Epidemiological Evidence

OP exposure in adulthood is still a global threat as exposure can occur: occupationally and to those living with someone

who is occupationally exposed; to those living near sites where OPs are manufactured or applied; and/or via ingestion of food and/or water contaminated with pesticide residues. Adult OP exposure has been associated with adverse neurological outcomes including neuropsychiatric conditions [106], cognitive deficits, and neurodegenerative diseases; for review, see [28, 43, 107, 108]. Some studies have failed to detect associations between occupational OP exposure and neurotoxicity, but this could be because the specific type of OP and individual susceptibility/sensitivity factors such as gene polymorphisms are rarely assessed [109]. Although many of the reported adverse outcomes (e.g., neuropsychiatric conditions such as depression; neurodegenerative diseases) also show sex-specific patterns [110], little attention has been paid to sexually dimorphic responses to adult exposure. Most studies of adult OP exposure focus on occupational exposure rather than indirect low-dose exposure, and yet while females comprise over 40% of the global agricultural workforce [111], a majority of studies on the adverse effects of occupational exposure examine effects solely in male participants or recruit women only to include them in the unexposed group, e.g., [26]. This creates non-comparability between the exposed and unexposed groups given all that we know about the underlying differences between female and male brains. However, some studies have indeed reported sex-specific effects of occupational OP exposure, which we will discuss below (and include in Appendix Table 1).

OP exposure determined by occupation, residues in house dust, and urinary DAP metabolites in migrant farmworkers ages 20–52 was associated with dominant-hand finger tapping and overall neurobehavioral performance in a sex-dependent manner. Similar to other epidemiological studies that have found significant sex-dependent motor effects [56], these deficits were greater in females than in males [112]. However, the sex-dependent effects on overall neurobehavioral performance should be regarded with caution as the metric was a summary index that excluded certain measures such as selective attention that have been found to impact males more than females; the validity and utility of such a summary index is unclear. One study conducted only on females also found motor deficits (longer reaction times, reduced motor steadiness) in greenhouse workers occupationally exposed to low doses of OPs compared to unexposed females [113]. However, exposure was classified using non-biological markers of exposure. Why female motor control would be more susceptible to OP exposure is unclear, especially because they are less sensitive than males in most of the other neurological outcomes affected by OPs.

Occupational exposures are generally higher than the doses most of the population is exposed to (e.g., through food and water contamination), and most studies of occupational exposure are cross-sectional in design, yet because agricultural work using pesticides is influenced by seasonality, current measures of exposure (such as urinary DAPs, for example) may not be indicative of average annual exposure. Clearly, more longitudinal research needs to be conducted on sex differences resulting from occupational OP exposures and low-dose exposures throughout adulthood as the literature is sparse.

Experimental Evidence

Sexually dimorphic outcomes in experimental studies of occupational exposure have been virtually ignored (for review see [19•]). One study in male and female Wistar rats treated with a single dose of malathion (250 mg/kg) in early adulthood (3 months of age) found decreased hippocampal AChE activity more pronounced in males [114]. Also, a sex-specific effect was seen for glutathione *S*-transferase (GST) and glutathione reductase (GR), enzymes that detoxify xenobiotics and aid in defending against oxidative stress [115]. In the hippocampus, GST decreased in males only, and in the cortex, both GST and GR decreased in males only [114]. While this study unfortunately did not measure any behavioral endpoints, such sex differences in antioxidant-related enzymatic activities (albeit in response to a high acute dose of malathion) could be a mechanism of the sexually dimorphic outcomes observed in humans and should be explored further.

Another study examining AChE activity in response to an acute OP dose obtained similar results; after mice (2–2.6 months of age) received a single sublethal dose (6.33 mg/kg) of diisopropylfluorophosphate (a surrogate of the nerve gas sarin), brain AChE activity returned to control levels in females after 20 days, whereas in males AChE activity never fully recovered but plateaued to 75–80% of control values up to 40 days after exposure [116]. Thus, sex differences in AChE activity [116] or paraoxonase 1 activity, a detoxifying enzyme, may play a role in differential neurotoxicity.

Conclusions

While the epidemiological evidence is still limited, there is nonetheless growing indication that males are more sensitive than females to most of the adverse neurological outcomes related to OP exposure throughout the life course, with the exception of some deficits in motor function preferentially affecting females [21, 56, 112]. In general, the effects of OP exposure in adulthood do not seem as detrimental as in earlier stages of development; it appears that the adverse effects of OP exposure are greater in magnitude the earlier in development the exposure occurs. However, this could be due to the outcome measures employed in the assessment of adults versus children and the fact that the dose and route of exposure likely change across the lifespan. Also, sex-specific effects do

not occur until after mid-gestation, when underlying sexual dimorphisms in the brain begin to form.

Interestingly, it has been shown that even in the womb, males may have higher rates of exposure than females [117•], an observation for which there is no obvious explanation. Perhaps the hormones associated with the development of a male or female fetus somehow modulate the detoxification capacity of the mother or increase her exposure via elusive behavioral changes which remain to be demonstrated. This differential sex-specific exposure in utero, if confirmed in other studies, should be investigated further as a potential mechanism underlying sex-specific differences in adverse outcomes. In addition, sex differences reported in pesticide-related hospitalizations among children (ages 5–14) and teenagers (ages 15–19) indicate that in addition to higher exposures in utero [117•], males have higher exposures after birth as well [118], possibly due to higher risk-taking behavior than females.

As highlighted in this review, only one isolated in vitro study has investigated sex-specific OP-related effects on brain cells [61••] and found some mechanistic insights supporting the preferential vulnerability of males to the deleterious effects of OPs at the cellular level. This finding is encouraging regarding the validity and the feasibility of this type of study; as previously discussed, it is not trivial to reproduce in vitro conditions optimal for the study of sexual dimorphisms. This is a new field of investigation that deserves more attention from both scientists and research product manufacturers.

As previously recommended by others [46], we stress here that in future epidemiological studies, sex should be analyzed as an effect modifier rather than a potential confounder so that sex-dependent outcomes in response to exposure can be detected. Future studies should place emphasis on the use of reliable individual biomarkers of exposure and assess exposure longitudinally, with measures taken before, during, and after cumulative exposure and neurobehavioral assessment. Such longitudinal studies are warranted to investigate whether observed sex-specific effects of OP exposure subside over time, whether damage remains indefinitely (as has been suggested [93]), or whether effects worsen as the cohort ages with cumulative exposure across the lifespan to OPs and other toxicants.

Overall, the outcome of exposure can differ for the same OP depending on (1) the species tested; (2) neurological outcomes assessed; (3) behavioral assays to measure that outcome; and of course (4) the dose, the route of administration, and the developmental stage that exposure occurs. In addition, while the entire OP class results in similar neurotoxic outcomes, the exact pattern and magnitude of several effects of their non-cholinergic mechanisms of action differ. This results in seemingly disparate effects on the same neurochemical and behavioral end points. The exact mechanisms of sex-dependent OP effects are not fully established, but it is likely the combination of altered morphology, neuropeptide and neurotransmitter signaling, and neuroinflammation, among other mechanisms.

Lastly, most studies examining the effects of OP exposure examine the effects of a single OP. However, exposure to OPs rarely occurs in isolation and is often a complex mixture of OPs and other pesticides [13]. A study examining the neurobehavioral effects of OPs and pyrethroids, a different pesticide class, found no significant adverse neurobehavioral effects in Thai children [119]. Likewise, OPs and pyrethroids were found to have opposing effects in infants [120]. These findings suggest that the behavioral effects of compound pesticides may negate each other and warrant further investigation of the effects across sexes of exposure to pesticide mixtures.

Originally, our interest in sex-specific effects of OP exposure stemmed in sex ratio differences in the incidence of several late-onset neurodegenerative disorders such as Parkinson's disease and ALS that have both been associated with potential exposure to OPs and a higher risk in males. In contrast with neurodevelopmental disorders like autism and ADHD that are more clearly associated with early OP exposures and higher risks in males, for late-onset neurological disorders, it is unclear whether they result from early prenatal/perinatal exposure, chronic exposure in adults, or continuous exposure across the life course. Improving understanding of the environmental etiology of these disorders warrants longitudinal biomonitoring and a study of the effects of chronic, low-dose OP exposure comparing adults (and animal models) of both sexes exposed and unexposed to OPs at different critical time windows and throughout the life course. The disappearance of sex ratios later in age could represent an initial research trail to follow: sexual dimorphisms in protective effects. While much is known about the adverse neurotoxic effects of OPs, our knowledge on the effects across sex is still extremely limited; we have a long journey before we can untangle the role of sexual dimorphisms in processes of sex-dependent neurological disorders.

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Compliance With Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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- • Of importance
- •• Of major importance

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Appendix Table 1. Epidemiologic studies investigating sex-specific neurological effects of organophosphate pesticides at different windows of exposure across the life course

Reference	Study Population	Exposure Assessment / Biomarker(s)	Endpoint(s) Measured	Outcome Assessment Test(s)	Age at Eval-uation	Results
Prenatal Exposure						
Marks et al., 2010 [40]	Salinas Valley, CA, U.S.[1]	Maternal urinary DAP levels, measured at two timepoints during pregnancy	Attention, Hyperactivity	Child Behavior Checklist (CBCL), NEPSY-II visual attention subtest, Conners' Kiddie Continuous Performance Test (K-CPT), Hillside Behavior Rating Scale	3.5 and 5 years	Significant association between prenatal OP exposure and attention problems and ADHD scores in 5-year old males only
Fortenberry et al., 2014 [50]	Mexico City, Mexico[2]	Maternal urinary 3,5,6-trichloro-2-pyridinol (TCP_Y) levels	Attention, Hyperactivity	Conners' Parental Rating Scales-Revised, Behavior Assessment System for Children-Parental Rating Scales, Conners' Continuous Performance Test	6-11 years	Trend for increased ADHD Index in relation to TCP_Y tertiles among males
Eskenazi et al., 2007 [35]	Salinas Valley, CA, U.S.[1]	Maternal and child urinary DAPs, maternal urinary malathion- and CPF-specific metabolites	Neuro-development	Bayley Scales of Infant Development, Maternal report using CBCL	6, 12, 24 months	No association between early OP exposure and CBCL attention problems
Zhang et al., 2014 [57]	Shenyang, China	Maternal urinary DAP levels, Questionnaire	Neuro-development	Neonatal Behavioral Neurological Assessment	3 days	Prenatal DAP concentrations inversely associated with NBNA scores; association stronger in female neonates
Horton et al., 2012 [51]	New York City, NY, U.S.[3]	Plasma CPF in umbilical cord blood	Working memory	Wechsler Scales of Infant Intelligence (WISC-IV)	7 years	Borderline significant interaction between prenatal CPF exposure and child sex; Males experienced a greater working memory deficit than females
Furlong et al., 2014 [53]	New York City, NY, U.S.[4]	Third trimester maternal urinary OP metabolites (dialkyl phosphates, diethyl phosphates, dimethyl phosphates)	Social-reciprocal behavior	Social Responsiveness Scale (SRS)	7-9 years	Possible association between diethyl phosphates and adverse SRS scores among males; No association between prenatal DAPs and social functioning

Millenson et al., 2017 [54]	Cincinnati, OH, U.S.[5]	Maternal urinary DAP levels, measured at two timepoints during pregnancy	Social-reciprocal behavior	Social Responsiveness Scale (SRS)	8 years	No association between prenatal DAPs and social functioning
Rauh et al., 2012 [3]	New York City, NY, U.S.[3]	Plasma CPF in umbilical cord blood	Brain morphology	Magnetic Resonance Imaging	7 years	Significant sex by exposure interactions in different cortical regions indicative of a disruption of normal sex differences; Inverse dose-response relationship of cortical thickness with CPF
Rauh et al., 2015 [55]	New York City, NY, U.S.[3]	Plasma CPF in umbilical cord blood	Tremor	Tests of motor function conducted by trained neuropsychologist and neurologist	9-14 years	Males significantly more likely than females to demonstrate clinically meaningful tremor on all measures of tremor; More highly exposed groups showed significantly higher proportions with clinically meaningful tremor
Silver et al., 2017 [56]	Fuyang, China	24 OPs and 6 OP metabolites measured in umbilical cord blood	Motor function	Peabody Developmental Motor Scales 2nd edition (PDMS-2)	6 weeks, 9 months	No significant findings at 6 weeks; Prenatal naled and CPF exposure associated with decreased motor function at 9 months, effects stronger in females than males
Childhood/ Adolescent Exposure						
González-Alzaga et al., 2015 [99]	Agricultural communities in El Poniente, southeastern Spain	6 child urinary DAPs, geographic information system (GIS) modeling	Neuro-psychological development	WISC-IV	6-11 years	Greater urinary DAP levels associated with poorer performance on the intelligence quotient and verbal comprehension domain; Estimated pre- and postnatal exposure associated with poorer processing speed score in males only
Suarez-Lopez et al., 2013 [100]	Floricultural communities in the Ecuadorian Andes (Pedro Moncayo County, Pichincha,	Erythrocytic AChE actvity	Overall neuro-development, Attention, Inhibitory control, Memory	NEPSY-II	4-9 years	Significant effect modification by sex in total neuro-developmental summary and specific domains: attention and executive functioning, memory and learning (associations stronger in males)

	Ecuador)[6]					
Rohlman et al., 2005 [101]	Hood River County and Lincoln County, OR, U.S.; Eastern NC and Wilkes County, NC, U.S.	Residence in agricultural or non-agricultural community, Parent's occupation	Neuro-behavioral performance (including Cognitive functioning, Motor coordination, Reaction time)	Behavioral Assessment and Research System (BARS), computerized; Object Memory, Purdue Pegboard, Visual Motor Integration, non-computerized	48-71 months (2-6 years)	Sex interaction in Finger Tapping and Match-to-Sample; male agricultural children (OR) performed significantly worse on finger tapping than non-agricultural males; male agricultural children (NC) had significantly longer latencies on Match-to-Sample than non-agricultural males
Eckerman et al., 2007 [102]	Nova Friburgo, Rio de Janeiro, Brazil	Rural vs. urban residence, Interview	Neuro-behavioral performance	BARS, computerized	10-18 years	Group effects differed by age; Young rural residents (10-11 years) showed more significant impairment compared to young urban residents; Similar results when analyzing using the exposure index (rather than residence) Males showed negative association between exposure index and behavior score, met criterion for hinted association for two measures
Adulthood Exposure						
Rothlein et al., 2006 [112]	Hood River County and Lincoln County, OR, U.S.	Urinary DAP levels during summer and fall, Analysis of home dust samples, Interview, Residence in agricultural vs. non-agricultural county	Neuro-behavioral performance (Psycho-motor functioning, Cognitive functioning)	BARS, computerized	19-52 years	Significant agricultural status by sex interaction found for preferred-hand finger tapping test and neurobehavioral summary index (non-agricultural females performed significantly better) Modest agricultural status by sex interaction for serial digit learning test, with non-agricultural females performing better (one-sided *p*-value=0.13)

[1]Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort

[2]Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study

[3]Columbia Center for Children's Environmental Health cohort
[4]Mount Sinai Children's Environmental Health Study
[5]Health Outcomes and Measures of the Environment (HOME) Study
[6]Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA: Estudio de la Exposición Secundaria a Plaguicidas en Infantes, Niños y Adolescentes) study

Appendix Table 2. *In vitro* and *in vivo* experimental studies investigating sex-specific central nervous system-related effects of organophosphate pesticides (excluding CPF)

Reference	OP	Model/ Species	Exposure Duration	Dose, Dosing Regimen	Route/ Vehicle	Eval-uation Period	Assay/ Measure	Results
In vitro studies								
Astiz et al., 2014 [61]	Dimethoate (DMT)	Cortical astrocyte cultures, derived from CD1 mice	24 hr.	2 µg/mL	Applied in culture	24 hrs. after exposure	Western Blot, Dihydro-ethidium (ROS[1] assessment)	In male cultures only, DMT increased inflammatory molecules, estrogen receptors, steroidogenic proteins, and aromatase mRNA levels; Estradiol decreased mRNA levels of some inflammatory molecules and reduced effects of increased ROS in male cultures
In vivo studies *Late gestation*								
Vatanparast et al., 2013 [70]	DZ[2]	Wistar rat	4 days (GD[3] 15-18)	1.0 mg/kg, daily	S.C.[4] injection, vol. 1.0 mL/kg, solubilized in DMSO[5]	PND[6] 60	Passive avoidance, Number of NOS[7]-containing neurons in basolateral complex of amygdala	Passive avoidance impairment in females only; No change in NOS-containing neurons
Naseh et al., 2013 [86]	DZ	Wistar rat	4 days (GD 15-18)	1.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	PND 4, 15, 60	Direct and indirect immuno-histochemistry (NOS-expressing neurons)	Main effects of gestational DZ on NOS-expressing neurons in cerebral cortex, hippocampus, and striatum were not sex-selective
Early postnatal								
Vatanparast et al., 2013 [70]	DZ	Wistar rat	4 days (PND 1-4)	1.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	PND 60	Passive avoidance, Number of NOS-containing neurons in basolateral complex of	Significant DZ by sex interaction, with males showing greater deficit; Large reduction in number of NOS-containing neurons in both sexes (i.e. Effects on NOS-containing neurons were sex-independent but depended on OP and exposure window)

							amygdala	
Naseh et al., 2013 [86]	DZ	Wistar rat	4 days (PND 1-4)	1.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	PND 7, 15, 60	Direct and indirect immuno-histochemistry (NOS-expressing neurons)	Main effects of early postnatal DZ on NOS-expressing neurons in cerebral cortex, hippocampus, and striatum were not sex-selective
Timofeeva et al., 2008 [90]	DZ	Sprague-Dawley rat	4 days (PND 1-4)	0.5 mg/kg or 2.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	Different assays assessed at different timepoints between 4-18 weeks	T-maze alternation, Locomotor activity, Prepulse inhibition, Radial arm maze	Sex dependency found only for prepulse inhibition, a measure of sensorimotor gating, at week 11-12: Females were unaffected by DZ while males' typical response was feminized
Roegge et al., 2008 [91]	DZ	Sprague-Dawley rat	4 days (PND 1-4)	0.5 mg/kg or 2.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	Started at PND 52	Tests of emotional function: Elevated plus maze, Novelty-suppressed feeding, Chocolate milk anhedonia, Porsolt forced swim	Significant DZ by sex interaction for elevated plus maze (DZ-treated males spent significantly more time in open arms than controls); Significant sex-dependent effect in novelty-suppressed feeding (shorter latency to eat in males); Borderline significant fluid type by sex by DZ treatment interaction for chocolate milk anhedonia; No exposure-related differences in immobility time in forced swim test
Slotkin et al., 2008 [92]	DZ	Sprague-Dawley rat	4 days (PND 1-4)	0.5 mg/kg or 2.0 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	PND 30, 60, 100	Serotonin 1A and 2 receptors, Serotonin transporter sites	Serotonin 1A (5HT1A) receptors: Males: Lower DZ dose produced sig. decrease in 5HT1A receptors; Females: Non-significant trend towards an increase in 5HT1A (opposite of males) Serotonin transporter (5HTT) sites: Females: Lower DZ dose resulted in small but significant increase in 5HTT sites
Slotkin et al., 2009 [71]	Parathion	Sprague-Dawley rat	4 days (PND 1-4)	0.1 mg/kg or 0.2 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized	PND 30, 60, 100	Serotonin 1A and 2 receptors, Serotonin transporter	Significant treatment by age by sex by region by measure interactions; overall, parathion caused biphasic effect (initial upregulation of receptors that peaked and diminished globally until emergence of

					in DMSO		sites	deficits at P100) Long-term consequences of early parathion exposure on serotonin system distinct from those of CPF
Levin et al, 2010 [93]	Parathion	Sprague-Dawley rat	4 days (PND 1-4)	0.1 mg/kg or 0.2 mg/kg, daily	S.C. injection, vol. 1.0 mL/kg, solubilized in DMSO	14-19 months, 20 months	Radial arm maze, Neuro-chemical evaluations	Significant sex-dependent effects persisting into beginning of senescence; Males showed greater effects of parathion on behavior and neurochemical parameters; Progressive parathion-induced neural decline with aging
Johnson et al., 2009 [68]	Methyl parathion	Sprague-Dawley rat	21 days (PND 1-21)	Incremental dosing regimen (low, medium, high dose groups, between 0.2-0.9 mg/kg)	Oral gavage (dissolved in corn oil, 0.5 mL/kg body weight)	Started at PND 36	Radial arm maze (Working and reference memory)	Working memory: Significant decrease in males only at medium and high dose of methyl parathion Reference memory: Errors significantly increased in males only, at all doses of methyl parathion
Oriel et al., 2014 [72]	DFP[8]	BALB/c and C57BL/6 mice	7 days (PND 4-10)	1.0 mg/kg, daily	S.C. injection, 1 mg/10 mL/kg, diluted in 0.9% saline	PND 4, PND 10, Adulthood (3-5 months)	Fear condition-ing, AChE[9] splice variants	DFP-treated males of both strains showed increased conditioned cued scanning; Significant DFP by sex by strain interaction for cued freezing (male BALB/c froze more); DFP eliminated stress-induced increase in AChE transcript expression in BALB/c but not C57BL/6 mice Different strains can show distinct behavioral and physiological responses to OPs
Late postnatal								
Levi et al., 2008 [95]	DFP	C57BL/6 mice	7 days (PND 14-20)	0.5 mg/kg, daily	S.C. injection, vol. 1mL/100 g, diluted in 0.9%	Adulthood (4-6 months)	Active avoidance (Aversive learning assay), Set-shifting task (Attention, Discrimin-ation learning)	DFP exposure caused significant sex-dependent changes in complex learning and cognitive functions in adulthood, but did not affect active avoidance learning; DFP-treated females showed greater impairments

Adulthood								
Maris et al., 2010 [114]	Malathion	Wistar rat	Acute (one dose at 3 mos.)	250 mg/kg	I.P.[10] injection, malathion dissolved in hydro-carbons	24 hrs. after exposure	Brain AChE activity, Brain GR[11] and GST[12] activity	Detrimental effect on antioxidant-related enzyme activities in hippocampus and cerebral cortex was greater in males (i.e. decreased AChE activity more pronounced in males and GR and GST activity decreased in males only)
Smolen et al., 1987 [116]	DFP	DBA/2, C57BL/6, C3H/2 mice	Acute (one dose at 60-80 days)	6.33 mg/kg	I.P. injection, DFP solubilized in saline	40 day follow-up after exposure	Brain AChE activity, Choline acetyl-transferase activity	Females regained control AChE activity levels; Male AChE activity plateaued at 75-80% of control values and remained at that level for up to 40 days after single exposure; Male C3H mice tended to return closer to control levels than the other two strains

[1]Reactive oxygen species, ROS; [2]Diazinon, DZ; [3]Gestational day, GD; [4]Subcutaneous, S.C.; [5]Dimethylsulfoxide, DMSO; [6]Postnatal day, PND; [7]Nitric oxide synthase, NOS; [8]Diisopropylfluorophosphate, DFP; [9]Acetylcholinesterase, AChE; [10]Intraperitoneal, I.P.; [11]Glutathione reductase, GR; [12]Glutathione *S*-transferase, GST