A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides

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ARTICLE INFO
Article history:
Received 25 May 2012
Received in revised form
19 September 2012
Accepted 25 September 2012
Available online xxx

Keywords:
Glucose homeostasis
Lipid metabolism
Oxidative stress
Endocrine disrupting chemicals
Neurodevelopment
Neurodegeneration

ABSTRACT
Organochlorine and organophosphate pesticides are compounds that can be detected in human populations as a result of occupational or residential exposure. Despite their occurrence in considerably low levels in humans, their biological effects are hazardous since they interact with a plethora of enzymes, proteins, receptors and transcription factors. In this review we summarize the cell and molecular effects of organochlorine and organophosphate pesticides with respect to their toxicity, with particular emphasis on glucose and lipid metabolism, their interaction with some members of the nuclear receptor family of ligand-activated transcription factors, including the steroid and peroxisome proliferator activated receptors that changes the expression of genes involved in lipid metabolism and xenobiotic detoxification. More importantly, evidence regarding the metabolic degradation of pesticides and their accumulation in tissues is presented. Potential non-cholinergic mechanisms after long-term low-dose organophosphate exposure resulting in neurodevelopmental outcomes and neurodegeneration are also addressed. We conclude that the mechanism of pesticide-mediated toxicity is a combination of various enzyme-inhibitory, metabolic and transcriptional events acting at the cellular and molecular level.

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1. Introduction
Pesticides constitute a diverse class of chemicals extensively used for prevention of harmful effects caused by pests. Among the large number of different pesticides those that are of particular concern are organophosphate (OPs) and organochlorines (OCs). Despite their structural dissimilarities, these two classes of pesticides result in neurological adverse effects through different mechanisms of toxicity. OPs mechanism of action involves mainly inhibition of acetylcholinesterase (AChE) resulting in synaptic accumulation of acetylcholine and excessive stimulation of cholinergic neurons. Nevertheless this mechanism cannot alone account for some other effects such as neurodevelopmental alterations following low-dose chronic exposure by pregnant women. In turn, OCs have affinity for the α-subunit of the voltage-dependent sodium channels in neurons, preventing their closing and resulting in repetitive firing of action potentials (Karami-Mohajeri and Abdollahi, 2011). Primary adverse neurological effects of OC insecticides also result from inhibition of GABAA and glycine receptors (Heusinkveld and Westerink, 2012).

The major part of OPs used contain the P=S moiety (Chambers, 1992) and includes compounds such as parathion, diazinon, malathion and chlorpyrifos. Among the most commonly encountered classes of OC insecticides are chlorinated derivatives of dichloro-diphenyl-ethane, such as DDT (dichloro-diphenyltrichloroethane). Detection of such compounds may occur in a wide range of biological matrices, including blood and hair (Tsatsakis et al., 2008a,b). OPs are subject to several biotransformation reactions following their entry to the body, whereas OCs accumulate in organ tissues, without essentially undergoing metabolic degradation, thus showing long half-lives. Due to their potential for short and long term hazardous effects, continuous biomonitoring of the levels of pesticides and their metabolites in humans is an essential step towards the evaluation of risk assessment and the prediction of adverse health effects in populations with either occupational or background environmental exposure to pesticides (Kavallakis and Tsatsakis, 2012; Tsatsakis et al., 2012). In this review we will summarize the major long-term biological effects of OP and OC pesticides that contribute to their toxicity, focusing on the mechanisms of action at a molecular and cellular level.

2. Effects of OP and OC pesticides on glucose and lipid metabolism
OPs and OCs affect cellular metabolism of carbohydrate and lipids and may lead to insulin resistance and impaired glucose
homeostasis (Karami-Mohajeri and Abdollahi, 2011). Regarding possible mechanisms of action, it is well documented that exposure to OPs disrupts glucose homeostasis leading to elevated serum glucose levels (Amanvermez et al., 2010; Everett and Matheson, 2010). The underlying mechanism is thought to involve increased exocytosis of insulin and glucagon granules mediated by enhanced free cytosolic calcium levels as a result of continuous activation of protein kinase C signaling following acetylcholine binding to M3 muscarinic receptors in pancreatic β cells (Gilon and Henguin, 2001; Persaud et al., 1989). Tolerance to this effect may develop due to down-regulation of these receptors or reduction in β cell sensitivity to glucose following prolonged stimulation by acetylcholine (Montgomery et al., 2008). On the other hand, induction of oxidative stress by OPs via imbalance of the oxidative status of β cells can reduce glucose-stimulated insulin secretion (Karami-Mohajeri and Abdollahi, 2011), resulting in increased serum glucose levels. However, this mechanism has been challenged by a few animal studies.

OCs do not follow the same metabolic pathways than OPs. Compounds such as DDE are persistent and remain in the body for a long time period. The presence of multiple chlorine atoms in their structure increases their lipophilicity and results in accumulation in adipose tissue. Several studies have explored the possible relationship between OCs concentration and obesity (Jung et al., 1997; Lee et al., 2011; Pelletier et al., 2002, 2003). p,p-DDE levels have been reported to increase the proliferative capacity of preadipocytes, whose replication is needed for the development of adipose tissue (Chapados et al., 2012). Mechanistically, estrogens receptors (ERs) and estrogen contribute to the development of obesity as they regulate some aspects of metabolism, such as glucose transport, glycolysis, mitochondrial activity and fatty acid oxidation. Thus, ligands for the ER (such as OC compounds) may play a role in the control of adipogenesis, weight gain and insulin levels. As ERs are expressed in preadipocytes, estrogens and other chemicals binding to ERs may contribute to an increased number of adipocytes during development (Casals-Casas and Desvergne, 2011).

DDE has been also associated with increased body mass index (BMI), increased triglycerides and decreased high density lipoprotein (HDL)-cholesterol levels (Lee et al., 2011). A significant positive relationship has been observed between OC concentration and BMI in athletes suggesting that the adipose tissue level influences the storage of OCs in the body (Pelletier et al., 2002). Jung et al. (1997) found a positive relationship between the percentage of body fat in workers occupationally exposed to β-hexachlorocyclohexane and the half life of this compound. There is evidence to suggest that lipid mobilization that occurs during weight loss of obese subjects on a hypocaloric diet may have an impact on health mediated by the release of OCs accumulated in adipose tissue as a result of adipocyte lipolysis associated with reduced fasting insulin levels (Chievrier et al., 2000; Imbeault et al., 2002).

Exposure to low concentrations of some OC pesticides has been associated with metabolic dysregulation (excess adiposity and dyslipidemia) leading to prediabetes conditions. Furthermore, insulin resistance associated with obesity and exhaustion of pancreatic β cells from overproduction of insulin may progress to diabetes (Lee et al., 2007a,b, 2011).

Consistent with these observations is evidence suggesting the direct interaction of pesticides with peroxisome proliferator activated receptors (PPARs), which are ligand-activated transcription factors belonging to the nuclear receptor superfamily. OCs tend to bind strongly to PPAR and thus activate a large battery of genes involved in lipid metabolism, leading to an increased lipid oxidation, decreased tryglicerides accumulation and changes in glucose metabolism. Mechanistically, once PPARs are activated by lipid ligands, they bind to RXR (Retinoid X Receptor) and then the heterodimeric transcription factor complex binds to specific DNA sequence response elements located in promoter regions of target genes involved in the regulation of adipogenesis (adipocyte proliferation and differentiation), intracellular lipid metabolism and storage, glucose homeostasis, insulin sensitivity and embryonic and fetal development (Casals-Casas and Desvergne, 2011; Lau et al., 2010). Thus, PPARs act as lipid sensors that play a role in organs with a high rate of fatty acid metabolism in order to adapt gene expression to a given metabolic status (Casals-Casas and Desvergne, 2011). In addition to PPARs, estrogen receptors (ERs) are also involved in the regulation of adiposity and obesity, so that OC, as ER ligands, might be mechanistically associated to obesity. Interestingly, PPAR/RXR heterodimers are capable of binding to estrogen response elements in target genes, suggesting that signal cross-talk between these two nuclear receptors may participate in the control of obesity (Lau et al., 2010).

3. Interaction with endocrine system

Several pesticides have been documented to affect the endocrine system in any stage of hormonal regulation, from synthesis to hormone receptor binding (Bretveld et al., 2007), resulting in reproductive and developmental adverse effects. The OP insecticide dichlorvos increases the apoptosis of Leydig cells in the offspring of pregnant rats (Zeng et al., 2009) and methoxychlor, an OC compound, also induces testicular apoptosis (Vaithinathan et al., 2010). Exposure to very low biologically relevant concentrations of dieldrin, an environmental OC, may affect the fetal Leydig cells reducing testosterone synthesis, which may have an effect on reproductive development and adult fecundity (Fowler et al., 2007). Decreased testosterone levels following pesticide exposure results in a reduction of inhibin B concentration, which further induces FSH and LH secretion (Damstra et al., 2002).

High concentrations of p,p-DDE functions as an inhibitor of 5α-reductase, the enzyme that converts testosterone to dihydrotestosterone (DHT) (Frye et al., 2011; Hodgson and Rose, 2006; Luccio-Camelo and Prins, 2011). DDE has also shown to enhance basal and FSH-stimulated aromatase activity in ovarian granulosa cells (Younglai et al., 2004). As pesticides are small lipophilic compounds, they can bind nuclear receptors causing perturbation or modulation of downstream gene expression. For example, OC pesticides such as lindane may act as androgens antagonist due to inhibition of DHT binding to the AR. The resulting antiandrogenic effects involve impaired development of testes, changes in testes histology, cryptorchidism and decreased fertility (Luccio-Camelo and Prins, 2011). Similarly, DDT isomers also exhibit antiandrogenic effects by reducing the binding of DHT to the AR in vivo.

Mechanistically unrelated OPs, on their own, are capable of interfering with the endocrine function by inhibiting the binding of thyroid hormones to their corresponding receptors. OPs also reduce the metabolism of oestriadiol and perturb its normal function by potent inhibition of cytochrome P450 (CYP450) enzymes (Symonds et al., 2006; Trankina et al., 1985), as a result of the binding of the active sulfur atom that arises from desulfuration in phase I metabolism (Hodgson and Rose, 2006). OPs such as chlorpyrifos are also able to inhibit adrenal steroidogenesis, thus affecting the hormonal status (Civen et al., 1977; Walsh et al., 2000).

Inhibition of AChE in the hypothalamus after OP exposure alters the rate of gonadotrophin releasing hormone (GnRH) secretion, ultimately affecting secretion of pituitary hormones that stimulate the gonads (gonadotropic hormones), such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Krsmanovic et al., 2012).
Brain cholinergic activity also increases putative secretion of prolactin (Findling and Tyrrel, 1991).

4. Pesticide metabolism and induction of phases I and II enzymes

OP pesticides such as diazinon and chlorpyrifos are metabolized initially by CYP450 enzymes to yield oxidative desulfuration products, where the sulfur atom in the P=S moiety is replaced by oxygen (Hodgson and Rose, 2006). The so called “oxon” metabolites are then hydrolyzed by phosphotriesterases among which the most commonly encountered is the paraoxonase-1 (PON1). The main CYP450s involved in the oxidation reaction of OP compounds are CYP2B6, CYP2C19 and CYP3A4 (Flaskos, 2012). The oxon forms are responsible for the acute neurotoxic effects that occur following high exposure to OP compounds. It is evident that genetic variations in the above mentioned xenobiotic–metabolizing enzymes may alter their catalytic activity and the concentration of oxon metabolites in either serum or target tissues. PON1 is one of the most extensively studied xenobiotic metabolizing enzymes, as two frequently encountered genetic polymorphisms in the coding region (PON1Q192R and PON1L55M) result in lower catalytic activity and reduced protein levels, respectively (Tsatsakis et al., 2009, 2011).

The notion that OP levels in biological fluids are associated with PON1 status has been adopted by various studies (Lopez-Flores et al., 2009; Tsatsakis et al., 2011; Singh et al., 2011). Significant interactions have been reported between OP exposure and thyroid function in individuals with lower PON1 activity (Lacasaña et al., 2010). However other studies have found no association between PON1Q192R Polymorphism and poor general or mental health in the general population (Rice et al., 2009).

Induction of several phase I and II xenobiotic–metabolizing enzymes occurs as a result of xenobiotic entrance in the body. Given that OC pesticides are small molecular weight compounds with lipophilic nature, they may activate either the aryl hydrocarbon receptor (AhR) or the pregnane X receptor (PXR) and consequently facilitate the expression of genes encoding for phase I and II enzymes. Agonistic and antagonistic effects of pesticides towards AhR and PXR have been reported, as well as inhibitory and increased metabolic activities for detoxification enzymes, particularly hepatic CYP450 isoforms (Abass and Pelkonen, 2012; Chan et al., 2009; Han et al., 2007; Das et al., 2008; Scollon et al., 2009). OC pesticides bind strongly to the AhR and induce transcription of CYP1 family enzymes and glutathione-S-transferases (GSTs) (Karami-Mohajeri and Abdollahi, 2011). Endosulfan, an OC insecticide, has the ability to stimulate the PXR and/or the constitutive androstane receptor (CAR) resulting in activation of the expression of CYP2B6 and CYP3A4 (Casabar et al., 2010). OC pesticides such as diafox induce expression of hepatic and extrapathic CYP450s in vivo and in vitro, notably CYP1A1, CYP2B, CYP2E1 and CYP3A, whereas methoxychlor possesses antagonistic activity towards AhR and downregulates CYP1A1 levels (Abass and Pelkonen, 2012; Chan et al., 2009; Das et al., 2008; Han et al., 2007; Scollon et al., 2009). Increased levels of CYP450 enzymes may further metabolize and bioactivate other xenobiotic substances, such as nitrosamines and polycyclic aromatic hydrocarbons, leading to the formation of toxic metabolites (Androutsopoulos et al., 2009).

Increased oxidative stress, as a result of an enhanced generation of highly reactive molecules and/or reduced capacity of the antioxidant system of the body, is a second biological effect caused by OP and OC insult. This leads to DNA damage and changes in the expression of genes encoding for CYP450s, such as UDP-glucuronosyl transferases and GSTs, as a feedback mechanism for immediate detoxification of potentially harmful pesticide metabolites (Karami-Mohajeri and Abdollahi, 2011).

5. Non-cholinergic effects and developmental neurotoxicity

It is well established that acute cholinergic neurotoxicity caused by OPs is mediated by the inhibition of AChE by their corresponding oxon products, resulting in accumulation of acetylcholine, which in turn causes overstimulation of muscarinic and nictinic receptors. Recent reports suggest an additional mechanism of neurotoxicity due to OP interference with normal neurodevelopment that appears to be independent of the cholinergic effects since they occur at concentrations below those affecting cholinergic transmission (Pope et al., 2005). Chlorpyrifos and diazinon elicit adverse effects on brain development at exposures lower than those required to inhibit AChE, with the adverse effects involving the parent compounds and not their oxon metabolites, which are responsible for AChE inhibition (Jameson et al., 2007).

Evidence from in vitro studies indicates that diazinon-oxon is capable of reducing the number of neurites, whereas chlorpyrifos-oxon inhibits DNA synthesis in gloma cell lines and disrupts glial cell differentiation by affecting the integrity of the microtubule network (Flaskos, 2012). Chlorpyrifos-oxon and diazinon-oxon are clearly more potent than their parent compounds in inhibiting neuronal and glial cell differentiation. However, such effects are not related to AChE inhibition, as the concentration of chlorpyrifos-oxon needed to activate CREB, (Ca2+/CAM response element binding protein), a transcription factor involved in brain development, is lower than the concentration required to inhibit AChE (Schuh et al., 2002). More specifically, chlorpyrifos and chlorpyrifos-oxon increase the phosphorylation of CREB, without affecting total CREB protein levels, and thus stimulate diverse signaling pathways within neurons that are critical for neurodevelopment and cognitive function (Schuh et al., 2002). This effect was demonstrated at nanomolar concentrations, which are much lower than those required to inhibit AChE, suggesting that it occurs in an AChE-independent fashion (Schuh et al., 2002).

On the other hand, the expression pattern of AChE variants plays a role in the developmental neurotoxicity of OPs. It has been proposed that non-enzymatic functions of AChE splice variants may be involved in the mechanisms underlying the developmental neurotoxicity of OPs (Jameson et al., 2007). AChE occurs in neuronal and non-neuronal tissues where it exerts non-canonical functions independent of the acetylcholine-hydrolyzing capacity, such as neuritogenesis, synaptogenesis, proliferation, cell adhesion, apoptosis, hematopoiesis, and osteogenesis (Xie et al., 2011). These non-catalytic functions have been ascribed to two AChE variants: the more abundant synaptic isoform (AChE-S, a membrane multimeric enzyme) and the soluble monomeric “readthrough” isoform (AChE-R). Although both isoforms are induced by neural injury or cholinesterase inhibitors, overexpression of AChE-S is associated with injury and enhances neurotoxicity, whereas AChE-R appears to promote repair and protect against neurodegeneration (Jameson et al., 2007; Xie et al., 2011).

In summary, the various OP pesticides differ in key mechanistic aspects involved in their neurodevelopmental toxicity. These compounds affect brain development after fetal and childhood exposures through mechanisms other than cholinergic overstimulation, particularly by targeting pathways involved in normal cell development and alterations in the expression and function of nuclear transcription factors that control cells replications, differentiation and apoptosis (Darn et al., 2003; Slotkin and Seidler, 2007).
6. OP and OC pesticide exposure and neurodegeneration

Exposure to OPs can produce some long-term neurotoxic consequences as a result of acute poisoning and after long-term exposure to subclinical doses of OPs. Irreversible AChE inhibition after acute OP exposure may produce brain damage due to cholinergic neuronal dysfunction and excitotoxicity. Cholinergic neurons damaged by high OPs doses might be responsible for persist- ent profound neuropsychiatric and neurobehavioral impairments, including memory, cognitive, mental, emotional, motor and sensory deficits (Chen, 2012; Kanavouras et al., 2011; Androustsopoulos et al., 2011).

Different studies have pointed out mitochondria as a likely target for neural degeneration caused by chronic low-level OP exposure. Alterations of ATP concentrations and changes in mitochondrial transmembrane potential have been observed following exposure to neuropathic OP compounds, suggesting mitochondrial dysfunction (Carlson and Ehrich, 1999). Mechanistically, OPs might result in elevated mitochondrial calcium uptake that impair complex I and complex II function and decrease electron transfer activities of cytochrome oxidase (complex IV). Collectively, these events adversely affect ATP synthesis. As a result of disruption of mitochondrial transmembrane potential, cytochrome c is released from mitochondria to cytosol and several caspases are activated, leading to apoptotic cell death (Binukumar et al., 2010; Kaur et al., 2007). The alteration in the mitochondrial calcium uptake and mitochondrial electron transfer enzyme activities may cause lipid peroxidation, an increase in protein carbonyl, enhanced 8-hydroxydeoxyguanosine formation, and protein and mtDNA oxidation. Moreover, chronic OP exposure has the potential of generating reactive oxygen species and/or impairing cellular antioxidant defense system, leading to oxidative stress. In summary, chronic low-level exposure to OPs impairs mitochondrial bioenergetics resulting in apoptotic neuronal degeneration (Binukumar et al., 2010; Kaur et al., 2007; Wani et al., 2011). This common mechanism may underlie the development of many neurodegenerative diseases.

Increasing evidence suggests that in addition to excessive cholinergic stimulation, OP compounds are also capable of inducing activation of glutamatergic neurons and generation of reactive oxygen and nitrogen species, leading to lipid peroxidation with dendritic degeneration of pyramidal neurons and ultimately neurodegeneration (Zaja-Milatovic et al., 2009). Although chlorpyrifos-oxon is about three orders of magnitude more potent than chlorpyrifos in inhibition of brain AChE activity, chlorpyrifos-oxon is only slightly more potent in inducing apoptosis. Thus, apoptosis may play a mechanistic role in chlorpyrifos neurotoxicity that may be independent of AChE inhibition (Caughlan et al., 2004).

Another neurodegenerative disorder is organophosphate-induced delayed polyneuropathy (OPIDP), a rare toxicity that typically occurs only after very large exposures to OP compounds causing severe cholinergic toxicity. The molecular target for OPIDP is considered to be an enzyme in the nervous system known as neuropathy target esterase (NTE) (Lotti and Moretto, 2005). This enzyme is localized to the cytoplasmic face of the endoplasmic reticulum, the starting point for the constitutive secretory pathway and transport of neuronal materials into axons (Read et al., 2009). NTE catalyses the decalciylation of endoplasmic reticulum-membrane phosphatidylcholine to soluble products, glycerophosphocholine and fatty acids. In the nervous system of susceptible vertebrates, neuropathic OPs will cause a transient loss of NTE’s phospholipase activity, which in turn causes endoplasmic reticulum malfunction and perturbation of axonal transport and glial–axonal interactions; with the distal parts of long axons being particularly vulnerable to loss of these support functions (Glynn, 2007). Proteins involved in axonal transport, especially those whose function depends on reversible phosphorylation, may have also a role in OP-induced neurodegeneration (Lockridge and Schopfer, 2010).

Long-term environmental exposure to certain OC insecticides (i.e., lindane and dieldrin) has been associated to the development of neurodegenerative disorders, such as Parkinson’s disease (Heusinkveld and Westerink, 2012). A combination of lindane and dieldrin can induce a rapid increase in the levels of intracellular reactive oxygen species, a decrease in mitochondrial membrane potential and activation of caspases. Both oxidative stress and mitochondrial dysfunction may result in dopamine neurotoxicity, suggesting that those pesticides may play a role in the multifactorial etiology of Parkinson’s disease (Sharma et al., 2010).

Oxidative stress induced by endosulfan has also been implicated in the neurotoxicity of this OC insecticide. Mechanistically, endosulfan can modulate the activities of stress-responsive signal transduction pathways including extracellular signal regulated kinases (ERK). These kinases can further activate mitogen-activated protein kinases (MAPKs) and then enhance c-Jun phosphorylation, which in turn increases activating protein-1 (AP-1) activity resulting in activation of antioxidant response element (ARE)-mediated transcription (Song et al., 2012). DDt also exerts toxic effects on neuronal cells by the stimulation of MAPKs (Shinomiya and Shinomiya, 2003).

7. Conclusion

Pesticides are chemicals with diverse biological toxic effects that act on multiple cellular targets. OP pesticides are metabolized by CYP450s to their active oxons that are further hydrolyzed by PON1 whereas OCs tend to accumulate in adipose fatty tissues for a long time period, before being release to the blood. There is evidence that substantiates the effect of pesticides on glucose and lipid metabolism as well as their effect on normal endocrine and hormonal function, via antagonism with hormonal-binding. In addition, binding of organochlorine pesticides to nuclear and cytoplasmic receptors affects the expression of an array of genes encoding for xenobiotic-metabolizing enzymes as well as for cell growth and differentiation. Due to their potential for short and long term hazardous effects, continuous biomonitoring of the levels of pesticides in humans is an essential step towards the evaluation of risk assessment and the prediction of pathology in populations either occupationally exposed to pesticides or with background environmental exposure.

Conflict of interest

There are none conflict of interest to declare with respect to this article.

References


