Molecular and clinical aspects of embryotoxicity induced by acetylcholinesterase inhibitors

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Abstract

Acetylcholinesterase inhibitors are widely used for a variety of medical, agricultural and public health purposes. Consequently, exposure is highly possible during lifetime. However, their systematic use raises concerns for the potential impact on the fetus and newborn since these substances may affect angiogenesis, the neonatal and maternal intensive care, neuroimmune function and response, mammary growth/lactation via cholinergic/non-cholinergic central and peripheral neuroendocrine pathways. New methodologies, neuroscientific technologies and research studies are needed to harness existing knowledge along with the proper management, availability for new acetylcholinesterase inhibitors, with stable pharmacodynamics and clinical outcomes.

Abbreviations: ACh (Acetylcholine), AChE (Acetylcholinesterase), AChEIs (Acetylcholinesterase inhibitors), ASD (Autism Spectrum Disorder), BuChE (Pseudocholinesterase), EDs (Endocrine disruptors), PIP2= phosphatidylinositol (3,4)-bisphosphate, PIP3 = phosphatidylinositol (3,4,5)-trisphosphate, PI3K = phosphatidylinositol 3-kinase, PDK1 = phosphoinositide-dependent kinase 1, Akt = (Protein Kinase B), mTOR = mammalian target of rapamycin, HIF1α = Hypoxia-inducible factor 1-alpha, VEGF = Vascular endothelial growth factor, IL-1β = Interleukin 1 beta, IL-1R1= interleukin 1 receptor type 1, iNOS = inducible nitric oxide synthase

Key words: Cholinesterase Inhibitors; Endocrine Disruptors; Fetus; Organophosphates; Parasympathomimetics; Toxicity
1. Introduction

Acetylcholine (ACh) is the neurotransmitter at neuromuscular junctions. It is synthesized in nerve terminals. Its postsynaptic action is regulated by cholinesterase, a powerful hydrolytic enzyme, that includes two types; acetylcholinesterase (AChE) found in nerves, muscles and red blood cell membranes and pseudocholinesterase (BuChE) found mainly in the liver (Colovic et al. 2013; Lionetto et al. 2013). In states of great AChE reduction/suppression, BuChE can functionally replace AChE, even though AChE is the principal hydrolytic enzyme in excitable tissues such as the brain, nerves and skeletal muscles. Acetylcholinesterase inhibitors (AChEIs) or anti-cholinesterases inhibit cholinesterase, increasing the level and length of ACh action. A variety of usages of AChEIs are common in medicine and agriculture. AChEIs traditionally used for medical purposes include organophosphates and carbamates. In diseases such as myasthenia gravis and Alzheimer’s disease, in which cholinergic function is deficient, carbamates/organophosphates provide therapeutic options due to high effectiveness as AChEIs (Giacobini 2000). AChEIs are also used as pesticides for the elimination of insects that pose a threat to public health, agriculture and gardening (Baltazar et al. 2014; Dolapsakis et al. 2001; Koutroulakis et al. 2014; Mehrpour et al. 2014; Tsitsimpikou et al. 2013; Zafiropoulos et al. 2014; Zaganas et al. 2013). Despite the fact that low toxicity compounds such as pyrethroids and novel insecticides are available, carbamates and organophosphates are still widely used (Kavvalakis and Tsatsakis 2012). In many cases, their toxicity depends also on genetic polymorphisms implicated in their metabolism (Androutsopoulos et al. 2011; Tsatsakis et al. 2011; Tsatsakis et al. 2009). Furthermore, highly toxic organophosphates, such as sarin, tabun, and VX, have been implemented as nerve gases at times of war and attacks by terrorists in the late 20th century (Vucinic et al. 2017). However, acute, recurrent, or chronic exposure to carbamates/organophosphates can be toxic (Okumura et al. 2003). Therefore, clinicians may expect a wide variety of clinical outcomes since an individual can be exposed not only to medical AChEIs but also to pesticides or a combination of them.

2. AChEIs

Depending on mode of action, AChEIs are classified as reversible, irreversible, or quasi-irreversible (pseudo-irreversible). In general, reversible inhibitors are the least dangerous with mainly therapeutic applications. Irreversible AChEIs are mainly used as agricultural pesticides with intermediate toxic effects. Quasi-irreversible AChEIs are
the most dangerous and have been used as chemical weapons/pesticides. Nevertheless, the potential hazard is not only related to the substance itself but also to the length of exposition. Reversible AChEIs (competitive/non-competitive), such as carbamate, quaternary or tertiary ammonium group, mostly have therapeutic applications in various diseases such as myasthenia gravis, Alzheimer’s disease, post-operative ileus, underactive bladder (detrusor underactivity; distigmine bromide with conflicting clinical benefit (Aggarwal and Zimmern 2016; Jiang et al. 2017)), glaucoma, as well as antidotes to anti-cholinergic overdose. Irreversible AChEIs are associated with toxic effects (Colovic et al. 2013; Shaikh et al. 2014). Following organochlorine pesticides banning, organophosphorus/carbamate use has recently increased; commonest used pesticides globally nowadays (Lionetto et al. 2013). Small amounts remaining in the environment after agricultural use have been detected in ground, foodstuffs, drinking water and sea life, posing a potential risk to human health. Consequently, people are unavoidably exposed via inhalation, skin permeation or ingestion of contaminated food. (Lionetto et al. 2013). Quasi-irreversible AChEIs have been used as flame retardants, pesticides or chemical weapons. It is probably more accurate to classify them as weak/short-acting or strong/long-acting. AChEIs and applications are summarized in table 1.

3. Toxic effects of AChEIs

3.1 Systematic toxic effects of AChEIs

Major effects of AChEIs are due to their action on the parasympathetic nervous system, causing slow heart rate, low blood pressure, bronchial hypersecretion/constriction, gastrointestinal hypermobility, and decreased intraocular pressure. Massive parasympathetic discharge usually occurs only with drug overdose, consumption of certain poisonous mushrooms (particularly the muscarine-containing Inocybe and Clitocybe species) or exposure to nerve gases. The so-called “SLUDGE syndrome” summarizes the effects of stimulation of muscarinic receptors. According to the mnemonic “SLUDGE”, increased cholinergic stimulation (cholinergic crisis) is characterized by salivation, lacrimation (tearing), urination, diarrhoea, gastrointestinal distress due to smooth muscle tone changes (cramping) and emesis (vomiting), as well as meiosis and skeletal muscle spasm. Exposure to organophosphates such as parathion, malathion, and diazinon, or nerve gas such as sarin causes “SLUDGE” through irreversible phosphorylation/deactivation of AChE, raising ACh levels. It has been postulated that inhibiting AChE activity by 50%-60% may result to weakness, headache, dizziness, nausea and salivation that commonly resolve within 1–3 days. At
a 60%–90% inhibition level, moderate-intensity symptoms are seen including diaphoresis, vomiting, diarrhea, tremors, ambulatory disturbance, chest pain, and cyanosis that may reverse within a few weeks. However, at a 90%–100% inhibition level, respiratory or cardiac failure occurs resulting to death. Animal studies have also shown that organophosphates may induce neurodevelopmental manifestations at exposures below the threshold for AChE inhibition (Lionetto et al. 2013).

3.2 Toxic effects of AChEIs on the developing nervous system and liver

AChE plays a significant role in the outgrowth of axons, synaptogenesis, migration of neurons, hemopoietin stress responses and cell apoptosis. The mechanisms underlying these important non-catalytic functions remain to be explored as such functions are at a large extent independent of the enzymatic ability to hydrolyze ACh. Interestingly though, in several tissues, alternatively spliced AChE variants appear to be involved. As such, R and S AChE splice variants take part in brains’ development and repair functions. The AChE-R isoform is selectively induced by injury been able to promote repair/protect against neurodegeneration. On the contrary, upregulation of the more abundant synaptic isoform, AChE-S results in increased susceptibility to neurotoxicity (Lionetto et al. 2013). It has been suggested that the non-enzymatic functions of AChE splice variants are target for developmental neurotoxicity of organophosphates (Jameson et al. 2007).

There have been observations of associations between gestational organophosphate exposure and deficits in human fetal growth/neurocognitive development in children (Lionetto et al. 2013; Rauh et al. 2011; Rauh et al. 2006; Rauh et al. 2012). The sensitivity of the fetus to a toxic agent depends on the developmental stage. There are three fetal developmental stages. During blastogenesis, in the first two weeks of pregnancy, the agent either kills the fetus or affects only a few cells and the fetus continues to develop. In the embryonic period (3-8 weeks), when organogenesis takes place, the fetus is most susceptible leading to major congenital malformations. During the fetal period (9 week-term), these agents can cause growth restriction, functional deficits, neurologic impairment or minor malformations. However, the nervous system remains vulnerable in all stages throughout development. Spontaneous abortion could be the result of a toxic agent at every gestational age. The stimulation of cholinergic receptors in target cells during a critical developmental period provides signals influencing cell proliferation/differentiation. Cholinergic stimulation is essential for the establishment of a cerebrocortical cytoarchitecture, and even transient interference with
Cholinergic input during development can produce developmental anomalies as well as permanent structural and behavioral damage (Hohmann et al. 1991). Cholinergic overstimulation at inappropriate timing causes premature cessation of neuronal mitosis, leading to shortfalls in cell numbers and deficient synaptic activity in animals (Navarro et al. 1989). Impact in forebrain size/cortical thickness in rats’ pups treated prenatally and in vitro suppression of the activity of the leading edges of the extending axons have been observed after exposure to methanesulfonfonyl fluoride, physostigmine, neostigmine and edrophonium (Byers et al. 2005). It has also been shown that cerebellar purkinje/granule cells are important targets for substances such as thiophene (Fonnum and Lock 2000). Furthermore, chlorpyrifos administered to developing rats in doses that do not evoke signs of overt toxicity, decreases DNA synthesis and causes shortfalls in cell numbers in brain regions enriched in cholinergic innervation and apoptosis during neurulation. Interestingly, chlorpyrifos also evokes noncholinergic disruption of cell development by interfering with cell signaling via adenylyl cyclase leading to widespread disruption not limited to cholinergic systems. For example, chlorpyrifos inhibits DNA synthesis in undifferentiated PC12 cells, which have relatively few cholinergic receptors. It elicits damage by both noncholinergic and cholinergic mechanisms extending from early stages of neural cell replication through late stages of axonogenesis and terminal differentiation, suggesting that vulnerability is likely to extend from embryonic period into postnatal life (Slotkin 1999). In conclusion, unlike classical teratology, in which the first trimester of fetal development is the most sensitive target for adverse effects of drugs or chemicals, brain development is likely to be affected by exposures ranging from early embryonic stage through adolescence. 

Apart from nervous system dysfunction through cholinergic activity, AChEIs seem to significantly impair liver biochemistry as shown from studies in pregnant women living in agricultural areas and exposed to organophosphate pesticides. During the second trimester of pregnancy there were increases in ALT levels and the AST/ALT ratio, suggesting subclinical hepatotoxicity (Cecchi et al. 2012). In addition, in one series studying pregnant rats, administration of quinalphos produced significant changes in hepatic ALT, ALP and serum ALT, AST, ALP and LDH activity along with hepatocellular changes in dams (Srivastava and Raizada 1999).

3.3 AChEIs toxicity through endocrine homeostasis disturbance

In the last twenty years chemicals that are termed endocrine disruptors (EDs) have attracted increasing scientific interest. EDs interact with the endocrine system in ways
which may disrupt or disturb the normal function of this system in humans and wildlife. EDs exert their influences mainly by interfering with natural hormones because they can bind to their receptors and mimic or inhibit their action. EDs may interfere with the synthesis, transportation metabolism and excretion of hormones affecting endocrine system through a plethora of specific mechanisms that can attack the hypothalamic-pituitary-gonad, thyroid, and adrenal axes at different levels; therefore, they can have various health consequences throughout lifetime. For example, parathion inhibits catecholamine secretion, increases melatonin synthesis and inhibits gonadotrophic hormones (Panuwet et al. 2008); malathion inhibits catecholamine secretion and binds to thyroid hormone receptors (Ishihara et al. 2003), chlorpyrifos antagonizes androgen activity (Kang et al. 2004), dichlorvos acts as weak androgen receptor antagonist (Andersen et al. 2002), fenitrothion acts as a competitive AR antagonist and estrogen inhibitor (Tamura et al. 2003), while diazinon has estrogenic effects (Manabe et al. 2006). Human fetuses, infants and children exhibit greater susceptibility to EDs than adults (Goldman et al. 2004). Much of the damage occurs during gametogenesis and early fetal development. However, effects may not become apparent until later in life. Moreover, fetuses and infants receive greater doses due to mobilization of maternal fat reserves during pregnancy and breastfeeding. Due to increasing evidence that certain endocrine-related disorders in children may occur as a result of exposure to many EDs, scientific bodies, such as the Endocrine Society, as well as governmental authorities have expressed concern for the impact of such chemical compounds on human health. However, further investigation is warranted to elucidate such adverse effects on humans, especially children, as research for most EDs is still lacking (Meeker 2012). Nevertheless, owing to this propensity both in intra and extra-uterine life, a wide range of adverse effects may result including potential long-term impacts on intellectual function and delayed effects on the function of the central nervous system. A growing body of toxicology animal data suggests that exposure to EDs are linked to male reproductive system disorders as well (Petrakis et al. 2017). Despite contrary reports (Fratric et al. 2017; Kalliora et al. 2018), several studies have connected exposure to specific EDs such as organophosphate pesticides with human congenital abnormalities such as testicular maldescent and hypospadias (Garcia-Rodriguez et al. 1996; Huang et al. 2006; Kalliora et al. 2018; Kristensen et al. 1997; Michalakis et al. 2014; Weidner et al. 1998) which together with poor spermatogenesis are considered signs of testicular dysgenesis syndrome. Testicular dysgenesis syndrome is considered to be the result of
embryonal programming/gonadal development disruption during fetal life that is increasingly common due to adverse environmental influences, mainly exposure to EDs (Mamoulakis et al. 2002; Mamoulakis et al. 2017; Skakkebaek et al. 2001; Virtanen et al. 2005). Furthermore, exposure during gestation and/or lactation to various pesticides including some organophosphates has also been reported to perturb reproductive system/activity of male rodents; effect on histopathology/weight of gonads/accessory glands, alterations in sperm counts, hormonal levels, and impaired fertility have been reported (Verma and Mohanty 2009).

4.1 AChEIs during gestation

According to the safety of different medications for use in pregnancy, the Food and Drug Administration has appointed five different categories: Category A (no known risk to human fetus in the first trimester), Category B (no known risk to animal fetus, but insufficient evidence about human fetus), Category C (adverse effects in animal fetus—not enough evidence about human fetus but potential benefit may warrant use in pregnant women despite potential risk to the fetus), Category D (evidence that adverse effects exist for human fetuses) and Category X (fetal abnormalities—should not be used during pregnancy). Most AChEIs belong in B/C category. Common conditions posing a clinical dilemma include glaucoma and myasthenia gravis. There is no consensus regarding the administration of AChEIs in such cases since both treatment withdrawal due to unknown fetal hazards in glaucomatous patients and good pregnancy outcomes in treatment with pyridostigmine/neostigmine in myasthenia gravis have been reported (Birks et al. 1968; Chaudhry et al. 2012; Hoff et al. 2007). There is only one case report of microcephaly attributed to pyridostigmine use but the mother was taking 4-8 times the recommended dose (Dominovic-Kovacevic A 2010). Nevertheless, caution is required since the pharmacologic treatment might need to be altered depending on disease severity or exacerbations (Ferrero et al. 2005). During labor, AChEIs may increase weakness due to respiratory insufficiency/gastric content aspiration, resulting in increased perinatal stress risk. Maternal expulsive efforts may be markedly reduced at the end of the labor, increasing the need for instrumental delivery. Requirements for AChEIs may be difficult to estimate during labor, because inadequate dosage may lead to several weaknesses and overdose may lead to cholinergic crisis. In addition, there is increased sensitivity to non-depolarizing neuromuscular blocking drugs, with the risk of prolonged neuromuscular blockade during and after general anesthesia. Placenta transfer may cause neonatal weakness. Conditions that threaten the mother’s health may
well impact the fetus as well. AChEIs molecules may cross membranes into the placenta entering the fetus, or cross the blood-brain barrier entering the central nervous system. Physiologic changes in pregnancy impact pharmacokinetic properties of AChEIs. Double maternal blood volume and renal blood flow, alteration of hepatic metabolism and decrease of intestinal tone and motility result in prolonged transit time, allowing greater drug absorption and enterohepatic cycling to occur, causing variations and adjustment difficulties of maternal medications. Practically any AChEI during pregnancy will reach the fetus. Since there is no consensus regarding their administration, the clinician must consider the advantages and disadvantages of such a treatment.

4.2 AChEIs effects on fetal growth and development

Since 1965, Ogi and Hamada stated that certain instances of human fetal death and malformations that occurred in rural Japanese areas were related to methyl parathion, a compound used at that time as part of the agricultural practice to control the rice stem borer (Ogi and Hamada 1965). Nowadays, due to the increased infiltration of pesticides in the environment, human exposure has become inevitable, not only in developing countries, where agricultural practices still rely widely on pesticides but also in developed ones where insecticides are more often used for public health purposes and food products and water are usually contaminated with pesticides. Early data reporting the teratogenic potential of organophosphorus insecticides in mammals showed that they can cause nervous system lesions (Baron and Johnson 1964) and skeletal abnormalities in chickens (Greenberg and LaHam 1969; Khera 1966; Khera and Bedok 1967; Khera et al. 1966; Khera et al. 1965). Moreover, in a research study using the organophosphats demeton and fenthion in mouse embryos, low incidence of major abnormalities (exencephaly, micromelia, cleft palate) observed with demeton or fenthion treatment which came in contradiction to the previous study on mice, where 14% cleft palate was obtained, with injections of methyl parathion in carboxymethyl cellulose (Tanimura et al. 1967). It was assumed that the difference in teratogenicity could be attributed to the solvent, the compound itself or the species of mice tested. According to the authors, teratogenesis did not appear to be directly related to cholinesterase inhibition as demeton, a potent AChEI resulted mainly in embryotoxic rather than teratogenic effects (Budreau and Singh 1973). This compound was later found to be teratogenic to mice but not to rats (Tanimura et al. 1967). In another study using phosphamidon in mice, subchronic treatment of with phosphamidon was more
embryotoxic than teratogenic. Significant effects were produced when the dams were treated during pregnancy and for 30 days before mating. Longer treatment, however, induced some resistance in the dams, thereby causing less embryotoxicity (Soni and Bhatnagar 1989). However, a recent study of dimecron on developing chick embryos, showed that the percentage of abnormal embryos gradually decreased with increasing time of exposure, suggesting that the level of abnormality in the embryos caused by the treatment was lethal, as significant abnormalities in relation to organogenesis and overall retardation in growth were noted in the insecticide-treated embryos. The liver and kidney were severely affected by the pesticide (Sahu and Ghatak 2002). The potential association between pesticide exposure and the appearance of some human congenital abnormalities (including among others musculoskeletal abnormalities; neural tube defects; urogenital and cardiovascular abnormalities) has been recently investigated in a comprehensive review concluding that a trend towards a positive association between environmental or occupational exposure to some pesticides such as organophosphates and some congenital abnormalities appears to exist (Kalliora et al. 2018). Major effects of AChEIs exposure on fetal growth/development are summarized in table 2 (Baron and Johnson 1964; Budreau and Singh 1973; Byers et al. 2005; Constantinescu et al. 2017; Dominovic-Kovacevic A 2010; Fonnum and Lock 2000; Greenberg and LaHam 1969; Hao et al. 2015; Kalliora et al. 2018; Khera 1966; Khera and Bedok 1967; Khera et al. 1965; Khera et al. 1966; Khera and Bedok 1967; Khera et al. 1965; Kuwabara et al. 2006; Noguchi et al. 2014; Ogi and Hamada 1965; Perera et al. 2003; Ridano et al. 2017; Sahu and Ghatak 2002; Slotkin 1999; Soni and Bhatnagar 1989; Srivastava and Raizada 1999; Tanimura et al. 1967).

On this wavelength, studies using large populations have reported that maternal–fetal transfer of organophosphate pesticides may be possible, resulting in pre-term and low-weight neonates (Wang et al. 2012). Cholinergic nerves play a significant role in controlling myometrium; ACh stimulates uterus contractions/dilates arterial supply (Papka et al. 1999). Therefore, inhibition of AChE can lead to overstimulation of cholinergic fibers, resulting in premature initiation of labor. Exposure of pregnant rodents to organophosphates results in teratogenicity/embryotoxicity, and fetal growth deficits. Adjusting rodent gestation stages to the ones of human (rodent day 6.5 equals to human week 2, rodent day 8 equals to human week 3, rodent day 15 equals to human week 8 and rodent day 15.5 equals to human week 9) (Otis and Brent 1954) some of the potential effects of organophosphates exhibited in rodent embryos may be linked to
human. Miyazaki et al reported that donepezil and physostigmine reduce perfusion/capillary density through reduction of interleukin-1β action and expression of vascular endothelial growth factor because donepezil inhibits phosphorylation of protein kinase B, a downstream kinase of PI3K (Miyazaki et al. 2012). The inhibition of PI3K/protein kinase B pathway along with inhibition of interleukin -1β pathway is depicted in figure 1. Moreover, in vivo studies have shown that impaired placental angiogenesis predisposes to fetal low flow circulation, causing asphyxia, preterm labor and delivery mainly through imbalance of uteroplacental perfusion (Su et al. 2015). It could be postulated therefore, that donepezil might affect uteroplacental perfusion with severe effects on pregnancy but this hypothesis needs further testing. Increasing level of chlorpyrifos in umbilical cord blood is associated with decreased birth weight and body length, shortened pregnancy, neural tube defects, anencephaly or spina bifida. (Perera et al. 2003). Negative effects on placental development are also associated with adult diseases later in life (Ridano et al. 2017). In a study on children, brain morphological changes were detected on magnetic resonance imaging in the group with high concentrations of chlorpyrifos in cord blood (Rauh et al. 2012). Pertaining to the effects of organophosphate pesticides on child neurodevelopment, epidemiological studies based on measurements of the parent compounds in blood or urine metabolites have reported associations with abnormal reflexes in neonates (Young et al. 2005), poorer mental development in toddlers (Eskenazi et al. 2007), and poorer working memory and intellectual quotient in children (Bouchard et al. 2011; Horton et al. 2012; Rauh et al. 2011). Additionally, studies that investigated associations between antenatal exposure to organophosphate pesticides and Autism Spectrum Disorder, have reported an increased risk of Autism Spectrum Disorder or Autism Spectrum Disorder-like symptoms in association with exposure to organophosphate pesticides (Philippat et al. 2018). It has been reported that children in the category corresponding to the highest 20% of maternal urinary levels of organophosphate metabolites during pregnancy show a 7-point decrease in full scale IQ compared with children of mothers in the lowest 20% (Bouchard et al. 2011). Additionally, gestational exposure to organophosphate pesticides produces similar actions to those of nicotine on neuronal α7-nicotinic ACh receptor, accounting for developmental alterations of brainstem vital centers in victims of sudden unexplained death (Lavezzi et al. 2015).
In a few animal studies marked sex-specific differences were observed in relation to pesticide used, administration time and postnatal period (Byers et al. 2005; Dam et al. 2000; Wang et al. 2000). Several parameters were investigated in exposed rats’ pups such as forebrain size, cortical thickness as well as coordination skills and open field response. In one study, rivastigmine and physostigmine caused significantly greater AChE inhibition in females than in males in the cerebral cortex, hippocampus and striatum but not in the periphery. Rivastigmine was more effective in antagonizing the scopolamine-induced spatial memory impairment in female than in male rats. Interestingly, ovariectomy did not affect the degree of enzyme inhibition by rivastigmine in any area, whereas orchidectomy completely abolished the difference in enzyme inhibition. It was hypothesized that a testicular hormone suppresses the effect of rivastigmine, by reducing the amount of drug reaching the brain or its interaction with AChE (Wang et al. 2000). To sum up, early data coming from rat and chicken embryos treated with AChEIs (organophosphorus insecticides) suggest that there may be a possible teratogenic effect (for example skeletal abnormalities in chicken embryos) but the main concern is the embryotoxic effect of these substances on the liver, kidney, neural system and heart. Later on, more data came to light in order to provide a molecular rational of these effects with numerous cytokines being affected.

4.3 AChEIs and lactation

Most AChEIs are compatible with breastfeeding such as alcuronium, vecuronium, pyridostigmine, suxamethonium. Atropine is compatible with breastfeeding but there is need for monitoring the infant for side-effects (drying of secretions, temperature elevation and disturbance of the central nervous system). The use of neostigmine should preferably be avoided if possible, especially in combination with atropine, due to potential side-effects. Galantamine is excreted in human milk. The effects of malathion were investigated after exposure of rats’ pups through maternal milk during lactation. Several biochemical parameters related to oxidative stress such lipoperoxidation and antioxidant enzyme activities as superoxide dismutase and catalase in brain, plasma and erythrocyte were evaluated. Findings suggested that malathion exposure during lactation induced cerebral alterations and oxidative stress in rat pups (Selmi et al. 2012). The effects of AChEIs uses in the nursing infant and animals result from increased ACh. In animals, systemic or central administration of AChEIs increases oxytocin secreted by neurohypophysis and lowers prolactin release secreted by the anterior pituitary (Ostrom 1990), affecting the mammary growth and lactation (Meites 1959).
Direct or indirect-acting dopamine receptor agonists, drugs which impair serotoninergic neurotransmission, gamma-aminobutyric acid-mimetic drugs, histamine H2-receptor agonists, cholinergic (muscarinic and nicotinic) receptor agonists, potentiate the prolactin depletion in concomitant use of AChEIs (Muller et al. 1983). Conversely, major prolactin-stimulating agents such as dopamine receptor antagonists or blockers, drugs enhancing serotoninergic neurotransmission, blockers of serotonin reuptake, H1-receptor agonists or H2-receptor antagonists eliminate the AChEIs induced prolactin depletion. In animals also, ACh stimulates a sustained reflex release of oxytocin, during suckling, through nicotinic and muscarinic receptors, producing multiple increases in intramammary pressure (Clarke et al. 1978). Through prolactin-lowering, AChEIs eliminate the transcription of casein mRNA and the subsequent synthesis of alpha-lactalbumin, the regulatory protein of the lactose synthetase enzyme system and may decrease thus the lipoprotein lipase activity in the mammary gland (Ostrom 1990). AChE inhibition evokes a multiplicity of effects at both hypothalamic and pituitary level through nicotinic and muscarinic receptors that are implicated in the regulation of hypothalamic and anterior pituitary hormone secretion. For example, diisopropylfluorophosphate increased serum adrenocorticotropic hormone (2.7-fold) and corticosterone (9.1-fold), while suppressing thyroid stimulating hormone, prolactin, luteinizing hormone, and growth hormone by up to 95%. Cholinesterase inhibition implicates other neurotransmitter pathways involved in thyroid stimulating hormone and prolactin suppression, such as somatostatin and dopamine, respectively (Smallridge et al. 1991). Data on the safety of different medications during lactation is freely available, at LactMed (http://toxnet.nlm.nih.gov/newtoxnet/lactmed.htm). In conclusion, most of the clinically used AChEIs are compatible with breast feeding. However, infants from people who are more likely to be exposed to agricultural AChEIs are more likely to suffer the toxic effects of these substances such as cerebral alterations and hormone imbalance.

5. Conclusions
AChEIs are widely used in medicine, agriculture and public health. Despite widespread use, few studies have thoroughly investigated potential embryotoxicity. Nevertheless, molecular and clinical aspects of embryotoxicity investigated in animal and human exposure studies have show that AChEIs are capable to induce several congenital malformations but the exact mode of action of each agent remains largely obscure.
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Figure legend

Figure 1: A graphical presentation of PI3K/Akt pathway and IL-1β pathway leading to angiogenesis inhibited by donepezil. Physiologically, when Akt is activated by phosphorylation from PDK1, can increase angiogenesis by two different pathways. The first one is found downstream, where Akt activates mTOR and then mTOR activates HIF1α by increasing its translation. HIF1α promotes gene expression of VEGF and thus leading to angiogenesis. The second one is by upregulation of IL-1R1, allowing more IL-1β to exert proangiogenic properties by the induction of iNOS. This stimulates vasodilation and vascular remodelling.
Table 1. AChEIs classes, main substances and applications

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<tr>
<th>AChEIs Class</th>
<th>Substances</th>
<th>Application</th>
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<tbody>
<tr>
<td><strong>Reversible</strong></td>
<td></td>
<td>Medical use (diagnosis/treatment):</td>
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<td>(competitive A-E, non-</td>
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<td>A: Neurodegenerative disorders (e.g. Alzheimer’s disease)</td>
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<td>competitive F)</td>
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<td>B: Glaucoma,</td>
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<td>C: Myasthenia Gravis</td>
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<td>D: Cardiac arrhythmias</td>
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<td></td>
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<td>E: Post-operative ileus, underactive bladder, antidote to anticholinergic</td>
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<td>overdose, analgesic and other medical uses</td>
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<td>F: Analgesic and other medical uses</td>
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<td><strong>Irreversible</strong></td>
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<td>Public health and agricultural appliances</td>
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<td>A: Insects that affect public health (e.g., mosquitoes, flies, cockroaches,</td>
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<td>ticks, fleas, and bedbugs)</td>
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<td>B: Agriculture and gardening (e.g., grasshoppers, aphids, caterpillars,</td>
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<td>rice insects, and stinkbugs)</td>
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<tr>
<td><strong>Quasi-irreversible</strong></td>
<td></td>
<td>Chemical weapons or pesticides:</td>
</tr>
<tr>
<td>(pseudo-irreversible)</td>
<td></td>
<td>A: Flame retardants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B: Pesticides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C: Chemical warfare (nerve gasses)</td>
</tr>
</tbody>
</table>

A: Donepezil, rivastigmine, tacrine, huperzine A, ladostigil, ungeremine and galantamine
B: Carbamate neostigmine, rivastigmine, demecarium, physostigmine and pyridostigmine
C: Ambenonium
D: Edrophonium
E: Carbamate, Quaternary or Tertiary ammonium group, Rosmarinic acid, lactucopicrin, F: caffeine, alpha-Pinene

A: Organophosphates (echothiophate, isoflurophate, diisopropylfluorophosphate –DFP, methanesulfonyl fluoride –MSF, metrifonate)
B: Malathion, parathion, diazinon, fenthion, trichlorfon, triazophos, and ethion

A: Triphenyl phosphate
B: Organophosphates (echothiophate, diisopropyl fluorophosphate, cadusafos, chlorpyrifos, dichlorvos, dimethoate)
C: Sarin, cyclosarin, soman, tabun, VX, VE, VG, VM
Table 2. Major effects of AChEIs exposure on fetal growth/development

<table>
<thead>
<tr>
<th>AChEI Agent</th>
<th>Species tested</th>
<th>Embryotoxic or teratogenic effect</th>
<th>Molecular disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanesulfonyl fluoride, physostigmine, Neostigmine and edrophonium (Byers et al. 2005)</td>
<td>Rat</td>
<td>↓ forebrain size and cortical thickness</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In vitro suppression of the activity of the leading edges of the extending axons</td>
<td>Unknown</td>
</tr>
<tr>
<td>Thiophene (Fonnum and Lock 2000)</td>
<td>Rat</td>
<td>↓ activity of cerebellar purkinje and granule cells</td>
<td>Unknown</td>
</tr>
<tr>
<td>Chlorpyrifos (Slotkin 1999)</td>
<td>Rat</td>
<td>↓ DNA synthesis and ↓ cell numbers in brain regions enriched in cholinergic innervation + apoptosis during neurulation</td>
<td>Unknown</td>
</tr>
<tr>
<td>Quinalphos (Srivastava and Raizada 1999)</td>
<td>Rat</td>
<td>Hepatocellular changes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pyridostigmine (Dominovic-Kovacevic A 2010)</td>
<td>Human (case report)</td>
<td>Microcephaly</td>
<td>Unknown</td>
</tr>
<tr>
<td>Methyl parathion (Ogi and Hamada 1965)</td>
<td>Human (retrospective exposure study)</td>
<td>Fetal death and malformations</td>
<td>Unknown</td>
</tr>
<tr>
<td>Organophosphorus (Baron and Johnson 1964; Greenberg and LaHam 1969; Khera 1966; Khera and Bedok 1967; Khera et al. 1966; Khera et al. 1965)</td>
<td>Chicken</td>
<td>Nervous system lesions and skeletal abnormalities</td>
<td>Unknown</td>
</tr>
<tr>
<td>Demeton and fenthion (Tanimura et al. 1967)</td>
<td>Mouse and Rat</td>
<td>Exencephaly, micromelia and cleft palate</td>
<td>Unknown</td>
</tr>
<tr>
<td>Demeton (Tanimura et al. 1967)</td>
<td>Mouse and Rat</td>
<td>Teratogenic to mice but not to rats</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pesticide</td>
<td>Species</td>
<td>Effect Description</td>
<td>Outcome</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Demeton (Budreau and Singh 1973)</td>
<td>Mouse</td>
<td>Mainly in embryotoxic rather than teratogenic effects</td>
<td>Unknown</td>
</tr>
<tr>
<td>Phosphamidon (Soni and Bhatnagar 1989)</td>
<td>Mouse</td>
<td>Subchronic treatment → more embryotoxic than teratogenic, Longer treatment → less embryotoxic</td>
<td>Unknown</td>
</tr>
<tr>
<td>Dimecron (Sahu and Ghatak 2002)</td>
<td>Chicken</td>
<td>Treatment is lethal. Significant abnormalities in relation to organogenesis and overall retardation in growth. The liver and kidney were severely affected by the pesticide</td>
<td>Unknown</td>
</tr>
<tr>
<td>Organophosphate pesticides (Kalliora et al. 2018)</td>
<td>Human (retrospective exposure study)</td>
<td>Musculoskeletal abnormalities; neural tube defects; urogenital and cardiovascular abnormalities</td>
<td>Unknown</td>
</tr>
<tr>
<td>Donepezil and physostigmine (Constantinescu et al. 2017; Hao et al. 2015; Kuwabara et al. 2006; Noguchi et al. 2014)</td>
<td>Human</td>
<td>Reduced perfusion and capillary density ↓ expression of interleukin-1β and vascular endothelial growth factor because donepezil inhibits phosphorylation of Akt</td>
<td>Unknown</td>
</tr>
<tr>
<td>Chlorpyrifos (Perera et al. 2003; Ridano et al. 2017)</td>
<td>Human</td>
<td>Decreased birth weight and body length, shortened pregnancy, neural tube defects, anencephaly or spina bifida</td>
<td>Unknown</td>
</tr>
</tbody>
</table>