Persistent organochlorinated pesticides and mechanisms of their toxicity

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A B S T R A C T

Persistent organic pollutants comprised of organic chemicals like polychlorinated biphenyls, dibenzo-p-dioxins, dibenzofurans and organochlorinated pesticides which have many characteristics in common. Once released in the environment they resist physical, biological, chemical and photochemical breakdown processes and thus persist in the environment. They are subject to long transboundary air pollution transport. They accumulate in the food chain due to their lipophilicity, bioaccumulation and biomagnification properties. Human exposure occurs through inhalation of air, ingestion of food and skin contact. Because most of them bioaccumulate and remain preferentially in fat, their long-term effects are still a matter of public health concern. They are condemned for health adverse effects such as cancer, reproductive defects, neurobehavioral abnormalities, endocrine and immunological toxicity. These effects can be elicited via a number of mechanisms among others include disruption of endocrine system, oxidation stress and epigenetic. However most of the mechanisms are not clear thus a number of studies are ongoing trying to elucidate them. In this review, the underlying possible mechanisms of action and their possible roles in adverse developmental and reproductive processes are discussed and where possible a linkage is made to some existing epidemiological data. Both genomic and nongenomic pathways are used to describe these effects. Understanding of these mechanisms will enable development of strategies to protect the public by reducing these adverse effects. This review is limited to persistent organochlorinated pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT) and its metabolites, hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH) and endosulfan.

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1. Introduction

Persistent organochlorinated pesticides are a structurally heterogeneous class of organic compounds composed primarily of carbon, hydrogen and several chlorine atoms per molecule. They share similar properties with other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/furans (PCDDs/Fs). Once released in the environment, they break down very slowly in air, water, soil and in living organisms and are thus subject to long trans-boundary air pollution (LTAP) transport where they are carried over long distances via the atmospheric transport and human activities. Even the body burden of the flocks of migratory birds is a sizeable contribution to LTAP (WHO, 2003). As OCPs bio-magnify through the food chain, consumers of food of animal origin such as fish, meat, milk and dairy products end up with high levels of exposure and due to slow biodegradation, OCPs accumulate in the body: for example DDT can remain in the body for 50 years, lifelong sequestrated into...
the soft tissue compartments (mainly the adipose tissues), from which they partition to the bloodstream into plasma or serum lipids and are actively secreted into breast milk as the main elimination pathway in mammals.

Human exposure begins during early prenatal and continues during the breast-feeding neonatal periods, which are critical stages for the development and differentiation of sensitive body organs and systems. In fact, these chemicals cross the placenta to the fetus and are secreted into breast milk (Perera et al., 2005).

In adults the dietary exposure route accounts for more than 90% of total organochlorine compounds (OCs) burden for the general population, while workers are exposed mainly through inhalation and skin contact. OCs are rapidly absorbed in the small intestine and enter circulatory system where they are distributed throughout the body and accumulate in body tissues with high lipid contents, with a continuous exchange between blood and tissues. As such these chemicals have been detected in human tissues such as blood (whole blood, cord blood, serum and plasma), adipose tissues (in autopsies and living subjects), breast milk, muscles and hair (Appenzeller and Tsatsakis, 2012; Covaci et al., 2002; Dewailly et al., 1999; Jakszyn et al., 2009; Mrema et al., 2011; Rappolt and Hale, 1968; Tsang et al., 2011; Tsatsakis and Tutudaki, 2004; Tsatsakis et al., 1998, 2008; Tutudaki et al., 2003).

Exposure to these persistent chemicals has been associated with health effects including cancer (Aronson et al., 2000; Cohn et al., 2007; Mathur et al., 2002; Recio-Vega et al., 2011), reproductive defects (Nicopoloulou and Stamanti, 2001) and behavioral changes (Zala and Penn, 2004). These effects are believed to be related to their ability to disrupt the functions of certain hormones, enzymes, growth factors, neurotransmitters and to induce key genes involved in metabolism of steroids and xenobiotics (Gourouni et al., 2008).

The mechanism of action involves toxicokinetic and toxicodynamic processes, at several levels between chemical exposure and the endpoints at individual level. Variation within humans can affect the endpoint at any toxicodynamic and/or toxicokinetic steps (Mortensen and Euling, in press). Direct, non/genomic mechanisms of toxicity involve perturbation of the homeostatic redox level(s) of biological compartments (oxidative stress) and consequent cellular death through apoptosis. Indirect, genomic mechanisms of toxicity involve permanent modification of the transcription of gene elements by interference at the epigenetic level of DNA functioning.

Since little is known on the relationship among events that result in OCs’ toxicity in humans (Mortensen and Euling, in press), a number of studies are currently ongoing to investigate mechanisms responsible for OCs’ toxicity. A better knowledge on how these compounds influence the development and progression of diseases is a crucial step towards addressing the emerging diseases and hence improving public health. In this review, we provide an overview on available information of possible mechanisms of toxicity of some selected OCs and where possible a linkage is made to epidemiological data. We summarize findings of epidemiological studies and both in vitro and in vivo experimental studies (Tables 1–3).

The main OCs for which we review available information are dichlorodiphenyl-trichloroethane (DDT) and its metabolites, hexachlorobenzene (HCB), beta-hexachlorohexachlorohepane (B-HCH) and endosulfan. These chemicals have been employed in agriculture and in public health as insecticides and biocides for several decades but were mostly banned between the ‘70 and ‘90s. Only a few active substances, essentially DDT is still used in tropical countries to control malaria vectors through indoor residual spraying (Bouwman et al., 2011) and lindane for treatment of lice and scabies (Engeler, 2009). So, most exposures derive from past uses and, in certain cases, are still significant because of the long persistence and biomagnification of these compounds.

2. Mechanisms of OCs toxicity

2.1. Endocrine activity

The endocrine system controls, balances and produces hormones and modulates their actions in the body through a network of activation and repression pathways at multiple levels of synthesis, secretion, transport, binding, action or elimination of natural hormones. Steroid hormones are involved in cell development, metabolism, protein synthesis, behavior, reproduction and development. These responses are elicited through binding of hormones to their specific receptors such as nuclear receptors (NRs). Among the members of NRs are estrogen receptors (ERs), androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), constitutive androstane receptor (CAR), rodent pregnane X receptor (PXR), mineralocorticoid receptor and thyroid hormone receptors (TRs). Aromatic hydrocarbon receptor (AhR) is a key regulator of the cellular response to xenobiotic exposure (Swedeborg et al., 2009). NRs and AhR are involved in the induction of xenobiotic metabolizing enzymes.

In the absence of ligands NRs and AhR stay in the cytoplasm or in the cell nucleus in a complex form (Picard, 2006). In the presence of ligands, receptor-ligand binding occurs, followed by translocation of NRs/AhR to the nucleus. In the nucleus, a complex of aryl receptor nuclear translocator (ARNT) protein and co-activators is formed. The complex binds to the specific AhR-DNA recognition site and finally induces transcription of target genes. The ligand binding triggers conformational changes that lead to dissociation of the repressive complex, the recruitment of transcriptional co-activators and receptor dimerization (Hankinson, 2005). NRs homodimerize or heterodimerize with retinoid X receptor and AhR dimerizes with ARNT leading to gene transcription of NR or AhR target genes (Claessens and Gewirth, 2004; Swanson, 2002). NRs control transcription through binding to promoter regions of DNA.

Several chlorine atoms in the molecular structures of OCs impart their highly lipophilic character and fairly rigid conformation (Fig. 2). The chlorine atoms are poorly reactive towards nucleophile displacement and elimination reactions and thus their biotransformation and biodegradation reactions are limited and are mostly confined, albeit to poor yield, only to anaerobic environment of sludges, but seldom in mammalian organisms. As a consequence, their interaction with biological systems is mostly limited to agonistic or antagonistic binding to the intracellular receptors for which natural hydrophobic substances, such as steroid derivatives, are the endogenous ligands. Agonistic binding leads to recruitment of coactivators and thus increases transcriptional activity while antagonistic binding prevents coactivator recruitment and/or attracts corepressors, leading to decreased transcriptional activity of the receptors.

Many POPs including OCs are known or suspected to be endocrine active. When present in the body they may interfere at several control points in the hormone signaling pathways. As a result, the response cascade of natural hormones can either be inhibited or excessively enhanced, at the wrong time, in the wrong tissue (Swedeborg et al., 2009). Endocrine activity of OCs can be due to direct binding with hormone receptors due to their conformational similarity with the receptor-binding portions of natural hormones, mainly of the steroid and diphenylether (thyroxine) structural groups. This is the case of aromatic polychlorinated substances such as DDT and its structural cognates, endosulfan and lindane. Other compounds indirectly alter hormonal pathway by directly inhibiting enzyme activities responsible for biosynthesis of the precursors of steroid hormones. In particular, the DDT analog, mitotane, a pharmaceutical drug which is used to suppress cortisol production also causes a sharp raise in plasma cholesterol levels.
Table 1
Summary for selected epidemiological studies showing the relationship of DDT/DDE exposure and health-related effects.

<table>
<thead>
<tr>
<th>Author</th>
<th>Effects assessed</th>
<th>OCP measured</th>
<th>OCP level (μg/L serum)</th>
<th>OCP level (μg/g lipid serum)</th>
<th>Results</th>
<th>Pval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohn et al. (2007)</td>
<td>Breast cancer</td>
<td>DDT</td>
<td>Tertiles &lt;8.09</td>
<td>Tertiles 1.00</td>
<td>OR, 95% CI 0.01</td>
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<td>8.09–13.9</td>
<td>1.1–1.8</td>
<td>2.8 (1.1–6.8)</td>
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<td>&gt;13.9</td>
<td>&gt;1.8</td>
<td>5.4 (1.7–17.1)</td>
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<tr>
<td>McGlynn et al. (2006)</td>
<td>Liver cancer</td>
<td>DDT</td>
<td>−</td>
<td>Quintiles &lt;0.265</td>
<td>OR, 95% CI 0.049</td>
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<td>0.265–0.382</td>
<td>1.00</td>
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<td>0.383–0.521</td>
<td>1.4 (0.7–2.6)</td>
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<td>0.522–0.787</td>
<td>1.4 (0.7–2.7)</td>
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<td></td>
<td>&gt;0.787</td>
<td>2.0 (1.1–3.9)</td>
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<tr>
<td>McGlynn et al. (2006)</td>
<td>Liver cancer</td>
<td>DDT adjusted</td>
<td>−</td>
<td>Quintiles &lt;0.265</td>
<td>OR, 95% CI 0.0024</td>
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<td>0.265–0.382</td>
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<td>0.383–0.521</td>
<td>1.7 (0.9–3.3)</td>
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<td>0.522–0.787</td>
<td>1.4 (1.0–4.3)</td>
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<td>&gt;0.787</td>
<td>2.0 (1.7–8.6)</td>
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<tr>
<td>McGlynn et al. (2008)</td>
<td>Testicular germ tumors</td>
<td>DDE</td>
<td>−</td>
<td>Quartiles &lt;0.157</td>
<td>RR, 95% CI 0.0002</td>
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<td>0.158–0.250</td>
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<td>0.251–0.390</td>
<td>1.00 (0.73–1.38)</td>
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<td>&gt;0.390</td>
<td>1.71 (1.23–2.38)</td>
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2.1.1. Interference of OCPs in estrogen hormone metabolism

17β-Estradiol is the main estrogen hormone, responsible for development of female sex characteristics such as growth of the breasts and suppression of body hair growth. Biotransformation of 17β-estradiol proceeds via two common metabolic pathways yielding catechol estrogen 2-hydroxyestrone (2-OHE), a weak estrogen; and 16α-hydroxyestrone (16α-OHE), a fully potent estrogen. Enzymes CYP1A1, CYP1B1, CYP1A2 and catechol-O-methyltransferase (COMT) are responsible for this metabolism. 16α-OHE, but not 2-OHE increases unscheduled DNA synthesis, uncontrolled cell division and increased anchorage-independent growth. Interrupting these pathways might lead to formation of more potent products as exemplified by some xenoestrogens. Exposure of cell cultures of MCF-7 to concentrations as low as 10⁻³ M of various OCPs such as DDT, lindane, chlordecone and endosulfan blocked the 2-OHE pathway and increase levels of 16α-OHE in estrogen positive (ER+) cells (Bradlow et al., 1995). This interference in estrogen biotransformation led to increasing 16α-OHE/2-OHE ratio relative to the ratio in the untreated control cell cultures. This increasing ratio has been associated with breast and other cancers of animals (Telang et al., 1992) (Fig. 1); possibly because of 16α-OHE has a strong estrogenic activity (Bradlow et al., 1995). Levels of 16α-hydroxylation have been found increased by about 50% in human breast cancer as compared to control cases (Schneider et al., 1982).

CYP1A1 metabolizes estradiol and environmental contaminants into the corresponding hydroxylated derivatives through the highly reactive intermediates of arene-oxide connectivity (Hayes et al., 1996). The expression of CYP1A1 is regulated by the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that activates the transcription of target genes, such as CYP1A1, CYP1A2, CYP1B1 and oncopgenes (Androutsopoulos et al., 2009b). DDT and its metabolites are capable of up regulating CYP1A activity (Van Tonder, 2011). According to Navas et al. (2004) CYP1A inducers have defined structures which make them capable of binding AhR. DDT has two aromatic rings linked by sp3 carbon and thus each of the rings can rotate along its single bond with the C2 carbon. This allows some degree of conformational mobility and in principle the possibility to bind AhR but the rings cannot lie in the same plane, as in the estrogen molecules (Fig. 3). Lack of this molecular recognition motif greatly limits the affinity of AhR for DDT. In fact, the concentration of DDT employed in the Bradlow experiments is 10⁻⁵ M (10 μM), while the physiological concentration of steroid hormones such as estradiol is of at least four to six orders of magnitude lower (low-picomolar). On the other hand DDT and its metabolites as well as many other OCPs are known phenobarbital-type cytochrome P450 inducers as they have been shown to induce cytochrome P450 2B (CYP 2B) in rat liver or cultured rat hepatocytes (Campbell et al., 1983; Li et al., 1995; Lubet et al., 1990, 1992; Yoshioka et al., 1984). The enzyme activation upon exposure to these pesticides can thus lead to alterations in the endogenous levels of hormones and consequently compromise hormone signaling. In addition to xenobiotics metabolism, CYP1A1 has been shown to participate in the activation of dietary polyphenols (Androutsopoulos et al., 2008, 2009a) and it is inhibited by various polyphenolics (Androutsopoulos et al., 2010, 2011).

2.1.2. DDT and DDE metabolites

DDT is produced as a technical mixture of 3 isomeric forms, the most prevalent being the p,p'-isomer; o,p'- and o,o'-being present in minor and variable amounts. DDT was initially used in public health control of lice and malaria mosquitoes during the Second World War and afterwards it was used as pesticide in agriculture. Globally DDT production was ~1.8 million tonnes since 1940s (ATSDR, 2002) and more than 40,000 tonnes were used in agriculture annually (Geisz et al., 2008). It is estimated 600,000 tonnes of DDT were used domestically in USA 30 years prior to its ban. Over 80% of the quantity of the pesticide used in 1970–1972 was applied to cotton crops and the remainder was used on peanut and soybean crops. The global production was reported to drop to 3314 tonnes in 2009; the production is meant for use in control of malaria and leishmaniasis (UNEP, 2010a).

DDT and its metabolite DDE exhibit hormonal activity in various tissues with mechanisms involving the steroidogenic pathway, receptor mediated changes in protein synthesis or antiandrogenic and estrogenic actions. Most of their endocrine effects result from the ability to mimic 17β-estradiol, o,p'-DDT is a well characterized DDT isomer; its intracellular mechanism of action is mediated through the ER pathway (Steinmetz et al., 1996) described in Section 2.1. It has been shown to bind and activate the ER, to promote the expression of estrogen-dependent genes and to induce proliferation of ER-dependent cells such as breast cancer cells (MCF-7).

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in vitro (Soto et al., 1995). At 10 μM concentration, o,p’-DDT agonized both ERα- and ERβ-mediated transcriptional potency that is approximately 0.1% that of estradiol (Lemaire et al., 2006). A significant dose-dependent increase in cell number in MCF-7 cell culture was observed starting at 10⁻⁸ M and maximum response achieved at 10⁻⁶ M (Steinmetz et al., 1996). Enantiomer-specific estrogenic activity has been suggested (Hoekstra et al., 2001; McBlain, 1987; Wang et al., 2009). At 5.26 × 10⁻⁵ M (S) (-)-o,p’-DDT produced half of the maximum effect (EC₅₀) whereas (R) (+)-o,p’-DDT had negligible estrogen activity (Hoekstra et al., 2001). The (+)-enantiomer exhibited estrogen-like response only at an environmentally realistic concentration of 10⁻³ M, and thus estrogenic activity of o,p’-DDT is due to the (-)-enantiomer. Transient cotransfection assay of monkey kidney CV1 cells with human AR expression vector and a mouse mammary tumor virus promoter with a luciferase reporter vector revealed that androgen-induced AR transcriptional activity was inhibited by p,p’-DDE or by the potent antiandrogen, hydroxyflutamide at 0.2 μM (63.6 ppb) concentration. This level of p,p’-DDE is lower than 140 ppb found among residents living in DDT-treated homes (Bouwman et al., 1991). In utero exposure to DDE has been shown to decrease anogenital distance (a morphologic quantitative indicator of feminization of male offspring) in male rat offspring, to increase retention of male nipple and to cause an alteration in expression of androgen receptor (Kelce et al., 1995) suggesting that these abnormalities might be mediated at the level of the androgen receptor. In addition to anti-androgen activity, p,p’-DDE can activate the ER and induce cell proliferation (Kelce et al., 1995). 17β-Estradiol (1 × 10⁻⁸ M) and β-HCH (1 × 10⁻⁵ M) similar showed gene expression profile of estrogen-responsive genes
<table>
<thead>
<tr>
<th>Author</th>
<th>Study type</th>
<th>Study sample/material</th>
<th>Effect investigated</th>
<th>Observed effects</th>
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<tbody>
<tr>
<td>Shi et al. (2009)</td>
<td>In vitro</td>
<td>Rat Sertoli cells from testes of 18 to 20 days old male Sprague-Dawley rats. The cells were incubated with 10, 30, 50 or 70 μM p,p’-DDE for 24 h</td>
<td>Effects of p,p’-DDE on apoptosis, FasL, caspase-3, caspase-8 mRNA, procaspase-3, caspase-8 and NF-κB activation.</td>
<td>Apoptotic cell death observed at &gt;30 μM; Fasl mRNA levels higher in a 50 μM group; caspase-3 mRNA levels higher in 30 and 50 μM group; caspase-8 mRNA increased in different doses; Fasl mRNA induced in 50 μM group, NAC attenuated this effect. Procaspase-3 and -8 significantly reduced over 30 and 10 μM, respectively; NF-κB activation enhanced with increase of dosage. No change in body weights and testis weights; selective degeneration of germ cells at the seminiferous tubules observed with &gt;20 mg/kg bw; all tested doses induced MDA increase and decrease SOD and CSH-Px activity; mRNA level of Fasl, Fas, caspase-3 and -8 elevated in 100 mg/kg bw group; p,p’-DDE induced increase in Fasl and reduction of procaspase-8; NF-κB p65 activated in 60 and 100 mg/kg bw groups; caspase-3 and 8 activities increased in 100 mg/kg bw group.</td>
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<tr>
<td>Shi et al. (2010)</td>
<td>In vivo</td>
<td>20-Day old male rats Prepupal rats administered with 0, 20, 60, 100 mg/kg bw of p,p’-DDE for 10 days of treatment</td>
<td>Effects of p,p’-DDE on body weights, organs weights, apoptosis, CSH-Px activity, MDA activity, Fasl, caspase-3 and -8; procaspase-8, NF-κB p65 proteins, caspase-3 and -8 activities in rat testis.</td>
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<td>Shi et al. (2011)</td>
<td>In vitro</td>
<td>Rat Sertoli cells from testes of 18-20 days old male rats treated with 10, 30, 50, 70 μM β-BHC to test the viability of cells. 50 μM p,p’-DDE was found to be the toxic concentration. The 10-30 and 50 μM β-BHC (β-HCH) were used in subsequent experiments</td>
<td>Effect of β-BHC on apoptosis, ROS production, LDL leakage rate, SOD activity, MDA level, JNK/p38 MAPK protein levels, Fasl, procaspase-3 and -8 protein levels, NF-κB activation, NF-κB p65 protein levels.</td>
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<td>Song et al. (2008)</td>
<td>In vitro</td>
<td>Rat Sertoli cells from testes of 18 days old male Sprague-Dawley rats. The cells were treated with 10,30, 50 or 70 μM p,p’-DDE</td>
<td>Effects of p,p’-DDE on LDLH leakage, ROS production, SOD activity and MDA level, ΔΨm, FasL, Bax family, cytochrome c translocation, procaspase-9 and -3 cleavage; effect of NAC on p,p’-DDE-induced apoptosis.</td>
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<td>Pérez-Maldonado et al. (2004)</td>
<td>In vitro and in vivo</td>
<td>Human PBMC from volunteers (without DDT exposure) for in vitro study. DDT exposed and unexposed children for in vivo study.</td>
<td>Effects of DDT or its metabolites on induction of apoptosis in humans both in vitro and in vivo.</td>
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<tr>
<td>Song et al. (2011)</td>
<td>In vitro</td>
<td>Rat Sertoli cells from testes of 18 days old male Sprague-Dawley rats treated with various 10, 30, 50 or 70 μM p,p’-DDE</td>
<td>Effect of NAC on p,p’-DDE-induced cellular viability reduction, ROS generation, MAPKs phosphorylation, effect of p,p’-DDE on mRNA levels of cytochrome c, bax, bak and bcl-w; effect of NAC on p,p’-DDE-induced apoptosis.</td>
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<td>Saradha et al. (2009)</td>
<td>In vivo</td>
<td>Adult male Wistar rats (80–90 days old) administered with a single dose of lindane (5 mg/kg b.wt)</td>
<td>The effects of lindane: on the levels of cytochrome c, caspase-3 and -9, Fas and Fasl; on localization of caspase-3 and Fas; on immunolocalization of Fasl; on levels of NF-κB p65 in the cytoplasmic and nuclear extracts of the testis, localization of NF-κB p65, bcl-w; effect of NAC on p,p’-DDE-induced apoptosis.</td>
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<td>Vaithinathan et al. (2010)</td>
<td>In vivo</td>
<td>Adult male Wistar rats (80–90 days old) administered with a single dose of methoxychlor (50 mg/kg b.wt)</td>
<td>The effects of methoxychlor: on the levels of cytochrome c, caspase-3 and -9 (in adult rat testis), Fas, Fasl, NF-κB (in testis cytoplasmic extract); on localization of caspase-3 and Fas; on immunolocalization of Fasl and NF-κB; on localization of NF-κB p65 in the rats testis; on germ cell in the testis of rats by TUNEL assay.</td>
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**Table 3:** Summary of recent studies on the apoptotic effects of OCPs (DDT, DDE, methoxychlor, β-HCH and γ-HCH).

**Abbreviations:** Caspases, cysteinyl aspartate-specific proteinases; CSH-Px, Glutathione Peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; NF-κB, Nuclear factor kappa B; LDH, lactate dehydrogenase; ΔΨm, mitochondrial membrane potential; MAPKs, mitogen-activated protein kinases; NAC, N-acetyl-cysteine; JNKs, c-Jun N-terminal kinase; PBMC, peripheral blood mononuclear; SE202190, p38 inhibitor; SP600125, JNK inhibitor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; ROS, reactive oxygen species; b.wt, body weight.
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**Fig. 1.** Bifunctional pathways to breast cancer (BCA). Abbreviations: OHE, hydroxyestrone; ER, estrogen receptor; GJI, gap junction inhibition; 17β-HSD, 17 beta-hydroxysteroid dehydrogenase. 17β-Estradiol metabolites affect cell proliferation and BCA development either directly via receptor-independent mechanisms involving structural/functional alterations in DNA, or indirectly via receptor-dependent mechanisms involving phenotypic growth regulation. These mechanisms upregulate aberrant proliferation and development of BCA.

Source: Adapted from Davis et al. (1998) with some modifications.

(i.e. TFF1, PR, ER, BRCA1 and CCND1) in MCF-7 cell line, while $p,p'$-DDE ($1 \times 10^{-5}$ M) was a weak transcriptional inducer (Silva et al., 2010).

In adult mice $p,p'$-DDT induced a dose-dependent effect on estrogen receptor activity in liver, brain, thymus and prostate of a transgenic mouse strain (ERE-tkLUC). The same effect was caused by $o,p'$-DDT, except in the liver. This study showed that DDT isomers modulate ER activity also in nonreproductive organs (Di Lorenzo et al., 2002). At a dose known to interfere with animal fertility (50 μg/kg), $o,p'$-DDT significantly affected ER response. Treatment of rats with 50 or 100 mg/kg body weight $p,p'$-DDT (repeated doses for ten successive days) induced decrease in the number and motility of epididymal spermatozoa in a dose dependent manner and DDT induction of testosterone metabolism causing reduced testosterone levels (Ben Rouma et al., 2001). The same treatment led to increased thyroid hormones and to a decrease in serum T4 levels and hypothyroidism (Tebourbi et al., 2010). Given the very high doses used in the experiments, the results of this study are hardly comparable with effects forecasted at human environmental exposure levels. In fact, a cross-sectional study conducted

**Fig. 2.** Molecular structures of phenobarbital (a prototypic CYP2B inducer) and some OCPs reviewed.

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among 781 young men did not find association between DDE levels (median: 2.7 µg/g serum lipid; range 0.1–56.1 µg/g serum lipid) and anogenital distance (Longnecker et al., 2007). Also a nested case–control study by Torres-Sánchez et al. (2008) among 37 young men showed that higher DDE levels (10th–90th percentile: 0.21–5.89 µg/g serum lipid) significantly reduced the anogenital distance. A study by Bornman et al. (2010) on urogenital malformations in male newborns suggests increased rates of malformations among those whose mothers lived in DDT-treated homes. Although these studies do not provide sufficient information for determination of a quantitative risk to humans, biological plausibility is strengthened by animal findings (WHO, 2011).

Cohn et al. (2007) conducted an epidemiological study and showed an association, with a dose response relationship, between DDT exposure and development of breast cancer when exposure is prepubertal. This was the case for the younger women (<14 years of age) in second and third tertile of exposure who had DDT exposure in their childhood (2nd tertile: 8.09–13.90 µg/L (1.1–1.8 µg/g lipid), OR = 2.8, 95% CI, 1.1–6.8; 3rd tertile: >13.90 µg/L, OR = 5.4, 95% CI, 1.7–17.1). McGlynn et al. (2006) found significant increasing risk of liver cancer among individuals with values in the highest versus the lowest quintile of serum DDT concentration (geometric mean = 0.49 µg/g lipids; OR = 3.8; 95% CI, 1.7–8.6; p for trend = 0.0024) when analysis was adjusted for age, sex, hepatitis B surface antigen status, places of residence and DDE level. The lowest and the highest quintile of serum DDT concentrations were <0.265 and >0.787 µg/g lipids, respectively. However there was no evidence of significant association between liver cancer and serum DDE concentration (p = 0.51). McGlynn et al. (2008) showed an association between testicular germ cell carcinomas and DDE exposure (upper quartile: >0.39 µg/g lipid, RR = 1.71; 95% CI, 1.23–2.38; Table 1).

Several epidemiological data showed an association between DDT exposure and miscarriage or preterm birth (Dewailly et al., 1993; Gladen et al., 2003; Karmaus and Zhu, 2004). To understand the involvement of the AhR/CYP1A1 pathway in the mechanism of action of DDT and DDE, Wójtowicz et al. (2011) used human placenta explants cultures originated from normal pregnancy and cotreated them with 3.2 ng/mL TCDD and 1, 10 or 100 ng/mL (approx. 0.18, 1.81, 18.10 µg/g lipid) of p,p’-DDT, o,p’-DDT, p,p’-DDE or o,p’-DDE for 24, 48 or 72 h (these concentrations are known to occur in serum of pregnant women). Immunoblot analyses showed that the isomers of DDT and DDE inhibited the expression of CYP1A1 most effectively at 48 and/or 72 h after treatment. In a separate experiment to study the expression of AhR, the placenta explants were cultured in the presence of these isomers at 100 ng/mL for 1, 3, 6, 14, 24, 48 and 72 h. The level of AhR protein decreased gradually with time starting after 3 h of treatment in all treatment experiments, with exception of that with o,p’-DDE, where the decrease was delayed at 24 h after the start of the experiment. The author argued that as CYP1A1 is involved in metabolism and detoxification in the human placenta, any malfunction of this enzyme will disrupt the placental detoxification machinery. This may lead to an increased susceptibility of the foetus to environmental toxins and may be a risk factor for recurrent pregnancy loss. A cross sectional study conducted by Tsatsakis et al. (2009) among a Greek rural population, revealed a significant association between CYP1A1*/2A polymorphism and miscarriages (p = 0.012). The investigated population had the median concentrations of 6.3, 2.9, 81.5, 3.1, 7.1, 5.3, 3.2 and 19.7 µg/g for α-HCH, HCB, lindane, o,p’-DDE, p,p’-DDE, o,p’-DDD, p,p’-DDD and o,p’-DDT, respectively. The significant associations were also evident for other ailments such as chronic obstructive pneumonopathy (p = 0.045), peripheral circulatory problems (trend p = 0.042), arthritis (p = 0.022), allergies (trend p = 0.046), hemorrhoids (trend p = 0.026) and allergic dermatitis (p = 0.0016). Thus it was suggested that CYP1A1 may play a significant role in the incidence of several diseases among the population with long exposure to organochlorinated pesticides.

1.3. Endosulfan

Endosulfan is synthesized via Dirlo–Alder addition of hexachloro-cyclopentadiene and cis-buten-1,4-diol in xylene. The annual production is estimated to range between 18,000 and 20,000 tonnes worldwide (UNEP, 2010b). In USA, 400 tonnes are estimated to be used annually for domestic purpose. The main uses include control of pests in vegetables, fruits, cereal grains and cotton, ornamental shrubs, trees, vines and ornamental plants. It was also used to control ectoparasite on beef and lactating cattle. In Africa it is commonly used in cotton production at a dose rate between 1000 and 2000 g/ha while in India it is used to control pests on cashew plantations at the dose rate of 1200 g/ha. The estimated annual production in India is 9500 tonnes, of which 4500–5000 tonnes are consumed domestically.

Most studies investigating endosulfan estrogrenicity in vitro show that its potency is 10^3–10^6 times lower than that of estradiol (Table 2; Arccaro et al., 1998; Soto et al., 1994, 1995; Wade et al., 1997). Brix et al. (2011) showed that endosulfan directly interacts with ERs in primary cultures of cortical neurons (CN) and cerebellar granule cells (CGC). It inhibited the binding of [³H]-estradiol to the ER in both CN and CGC with a fairly high IC_{50} of 21.7 ± 4.2 and 35.0 ± 8.3 µM, respectively. These IC_{50} values are very close to that of α-endosulfan with recombinant human steroid receptors particularly hPR (20 µM) (Scippo et al., 2004). Exposure of endosulfan at 10 µM for 5 h increased Akt phosphorylation and activated ERK1/2 through a mechanism involving GABAA and glutamate receptors in CN. The observed alterations on ER-mediated signaling and ER levels in neurons were suggested to contribute to the neurotoxicity of endosulfan.

In vitro assay showed that endosulfan, dieldrin and lindane act as antagonists of androgen receptors (Andersen et al., 2002; Li et al., 2008; Nativelle-Serpentini et al., 2003). Endosulfan and dieldrin were shown to inhibit the aromatase enzyme (CYP19); the rate-limiting enzyme of estrogen synthesis from the non-romatic precursor androstenedione. Like other organochlorines, endosulfan and dieldrin alter the estradiol metabolism by inducing CYP1 enzymes (Badawi et al., 2000; Bradlow et al., 1995). Endosulfan and dieldrin were shown to inhibit androgen response at 20 µM and thus acted as very weak antiandrogens. At 50 µM endosulfan reduced the aromatase activity in human placenta microsomes to 87% of the control level (i.e. 1 µM of 4-Androstened-4-ol-3,17-dione) and hence exhibited weak aromatase-inhibiting effect (Andersen et al., 2002).

In vitro assay shows that dieldrin (5 µM) and endosulfan (1 µM) significantly increase cell proliferation and ER transactivation gene response in MCF-7 cells (Andersen et al., 2002). The maximum response occurred at 25 µM for both dieldrin and endosulfan. However, only endosulfan was shown to potentiate the 17β-estradiol...
induced proliferation when tested together with a concentration of 17β-estradiol causing a sub maximum response. Other authors have also shown the ability of endosulfan to promote MCF-7 cell proliferation in vitro (Grunfeld and Bonefeld-Jorgensen, 2004; Ibarluzea et al., 2004; Soto et al., 1994). However, there are no epidemiological studies linking exposure to endosulfan and increase of cancer risk in humans. In vitro studies examining the effects of endosulfan on androgen-responsive systems showed that endosulfan exhibited cytotoxic effects on testicular cells at a concentration of 20 μM or greater (Sinha et al., 1999, 2001a). These doses are environmentally irrelevant. In vivo assays have not demonstrated estrogenic effects (Gellert, 1978; Raizada et al., 1991; Shelby et al., 1996; Wade et al., 1997). Endosulfan caused alterations in testicular functions at very high doses. At doses of 2.5, 5 and 10 mg/kg administered orally to adult Druckrey rats for 5 days per week for 70 days, endosulfan was shown to increase testicular enzyme activities at all tested doses. Sperm count decreased in a dose-dependent manner (Sinha et al., 1995). Similar study conducted for 90 days using weaning Druckrey rats yielded similar results where spermatid count and sperm production rate decreased (Sinha et al., 1997). Repeated dose of 3 mg/kg/day of endosulfan administered to immature rats for 3 days caused no change in uterine weights, uterine peroxidase activity, or numbers of uterine estrogen and progesterone receptors. Only at a high dose (10 μM) endosulfan inhibited estradiol binding (Wade et al., 1997) as was the case in for in vitro studies discussed above.

Exposure of adult rats to 2.5, 5 or 10 mg/kg endosulfan for 10 weeks (5 days per week) or 3, 6 or 1000 mg/kg bw/day endosulfan have shown to cause reduction in intratesticular spermatid counts, sperm abnormalities, and changes in the marker enzymes of testicular activities, such as lactate dehydrogenase, sorbitol dehydrogenase, γ-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase (Khan and Sinha, 1996; Sinha et al., 1995). Moreover, exposure of pregnant rats to endosulfan at 1 mg/kg/day from day 12 through parturition has shown to decrease spermatogenesis in offspring (Sinha et al., 2001a). It should be noted that these doses are orders of magnitude higher than those to which humans are usually exposed in the living environment and at the workplace.

Limited human data exist on the effect of endosulfan. A cohort study conducted by Saiyed et al. (2003) among male school children (10–19 years old) showed that endosulfan exposure was associated with delayed male sexual maturity and interfered with the male sex-hormone synthesis. Sexual maturity rating (SMR) scoring for development of pubic hair, testes, penis, and serum testosterone level was positively related to age and negatively related to aerial exposure to endosulfan (AEE; p < 0.01). Serum LH levels were significantly positively related to AEE after controlling for age (p < 0.01). Serum endosulfan levels were significantly higher (p < 0.001) in the exposed population (mean 7.47 ± 1.19 ppb) than controls (1.37 ± 0.40 ppb). Moreover, a big proportion of exposed group (78%) had detectable serum endosulfan as compared to 25% of the controls. Although these findings match those expected on the basis of experimental evidences, nevertheless, it was not clear if endosulfan was the only chemical that could be linked to the observed associations.

Several cellular and molecular mechanisms of endosulfan toxicity have been proposed. These include mitochondrial dysfunction, induction of oxidative stress, modulation of activities of stress-responsive signal transduction pathways, activating protein-1 (AP-1) and antioxidant response element (ARE)-mediated transcription. Recently a model has been proposed in which endosulfan is shown to increase extracellular signal regulated kinases (ERK) 1/2 and p38 activities and, in turn, these proteins activate mitogen-activated protein kinases (MAPKs) and increase c-Jun phosphorylation. Phosphorylated c-Jun, in turn, increases AP-1 activity, which results in activation of ARE-mediated transcription (Song et al., 2012). However, at the moment no firm conclusion can be drawn on these proposed mechanisms.

2.1.4. Hexachlorobenzene

Hexachlorobenzene, a fully chlorinated aromatic hydrocarbon, was widely used as fungicide. Its half life in human is estimated to be in the range of 6 years (To-Figueroa et al., 1997). The most sensitive target organs are the liver, the ovary and the central nervous system (ATSDR, 2002). HCB is well known to induce porphyria through a free-radical generation mechanism (Mazzetti et al., 2004), due to its rather unique chemical characteristics of lipophilicity and possibly to conversion into the redox-active tetrachloro-1,4-benzoquinone. HCB is a weak agonist of the AhR protein (Hahn et al., 1989). Unlike estrogen, HCB does not have a significant affinity for the ER thus suggesting other intracellular pathways than the one taken by endogenous estrogen in mediating these actions.

HCB can elicit tumorigenic activity through AhR-dependent and independent pathways. In vivo administration of HCB has shown to induce alterations in insulin-like growth factors (IGFs) signaling pathway in mammary gland and mammary tumors in rat only when co-administered with N-nitroso-N-methylurea (Randi et al., 2006). Since the latter compound is a strong mutagen, HCB plays the role of a tumor cocarcinogen in rat mammary gland, as an inducer of cell proliferation and of c-Src kinase activity in MCF-7 breast cancer cells.

García et al. (2010) used ERα (+) MCF-7 and ERα (−) MDA-MB-231 cell lines to investigate ability of HCB to promote cell proliferation and alter insulin/IGF-I signaling pathway. At 0.005 and 0.05 μM, HCB significantly increased the proliferation of only MCF-7 cell lines, with no effect at 0.5 and 5 μM. In estrogen-depleted medium, HCB stimulated proliferation of MCF-7 cells only at the lower concentrations showing that the effect of HCB on cell proliferation was not dose responsive. Thus HCB stimulates proliferation in estrogen-sensitive cells in an ERα−dependent manner. At 0.005 and 0.05 μM, HCB increased the level of IGF-IR and IR in ERα (+) MCF-7 cell line. However at 0.5 and 5 μM, HCB did not alter the level of these receptors but increased CYP1A1 gene expression and induced apoptosis in MCF-7 cell lines, suggesting HCB effect on apoptosis is AhR-dependent. At 0.005 and 0.05 μM of HCB did not induce the expression of CYP1A1 mRNA. On the other hand 0.005 μM of HCB led to c-Src activation signifying this effect is not AhR-dependent. At 0.005, 0.05, 0.5 and 5 μM, HCB increased IRS-1 phosphorylation with no effect in IRS-1 protein levels. IGF-1 receptor is known to regulate proliferation, survival and differentiation in mammary gland. Insulin-like growth factor-insulin receptor (IGF-IR) pathway is involved in development of breast cancer and IR content is known to increase in human breast cancer whereas IRS-1 is the main intracellular substrate activated by IGF-1 and insulin in human breast cancer cells. c-Src can promote growth of tumor cells, participating in or augmenting mitogenic signaling pathways that are initiated by extracellular growth factors or intracellular oncogenes (Biscardi et al., 2000). As per authors’ point of view, these results give a clue to the molecular mechanism of HCB in breast cancer development.

To investigate the effects of HCB, Pontillo et al. (2011) showed that at 0.05 μM HCB produced an early increase of c-Src, human epidermal growth factor receptor (HER1) activation, signal transducers and activators of transcription (STAT) 5b, ERK1/2, but not Akt, in ERα (−) MDA-MB-231 cell lines in a dose-dependent manner (0.005, 0.05, 0.5, and 5 μM).

Young rats (65 days of age) were administered 100 mg/kg b.wt of HCB three times a week (total dose of 1800 mg/kg b.wt over 45 day period) (Peña et al., 2012). When the test animals reached oestrous phase they were sacrificed. In mammary glands, HCB increased c-Src and HER1 activation, c-Src/HER1 association; and STAT5b and
ERK1/2 phosphorylation. Moreover HCB enhanced ERα phosphorylation and ERα/c-Src physical interaction. In tumors, HCB induced c-Src and HER1 activation, c-Src HER1 association, as well as Akt and STAT5b phosphorylation. Additionally, HCB increased ERα protein content and decreased p-ERα levels and ERα/c-Src association. HCB increased serum 17β estradiol and prolactin contents and decreased progesterone, FSH and LH levels in rats without tumors. This might be a consequence of a negative feedback on the pituitary hormones secretion, by E2. The opposite effect was observed in rats with tumors. These results indicate that HCB induces an estrogenic effect in mammary gland, increasing c-Src/HER1 and ERα signaling pathways. HCB stimulates c-Src/HER1 pathway, but decreases ERα activity in tumors, appearing to shift them towards a higher malignancy phenotype. The enhanced c-Src and HER1 activation could be associated to the increase in the hyperplasia found in the mammary gland and the higher malignancy observed in tumors (Peña et al., 2012). The synergism between c-Src and HER1 serves to upregulate the mitogenic activity of HER1 downstream effectors involved in tumorigenesis (Biscardi et al., 2000). However, the very high doses tested significantly affect the relevance of this information for human environmental and occupational risk assessment.

2.1.5. β-Hexachlorocyclohexane

Complex and variable mixtures of hexachlorocyclohexane (HCH) products are produced by photochemical chlorination of benzene. HCH exists in 9 stereoisomeric forms, which include (+)- and (−)-α- (α), beta- (β), gamma- (γ), delta- (δ), epsilon- (ε), zeta- (ζ), eta- (η) and theta- (θ) isomers. Technical-grade HCH is comprised of 60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH. Purified HCH isomers, as well as technical-grade HCH are used either as fungicides or in the synthesis of other chemicals.

Lindane (γ-isomer) was used as seed treatment for barley, corn, oats, rye, sorghum and wheat to protect seeds for sowing from molds and ants during storage before sowing and in the ground before sprouting. The estimation of global production between 1950 and 2000 was 600,000 tonnes and the majority was used in agriculture. In the past, γ-HCH was also used in veterinary products to control mites, lice and other pests, but recent data suggest that no products are currently registered, at least in the United States for this use. It was also used as an insecticide to treat fruit, vegetables, forest crops, animals and animal premises. It is also currently used in treatment of scabies and lice in humans in form of lotion, cream or shampoo, especially in tropical countries.

Another important HCH isomer is the beta-isomer (β-HCH). This isomer has all chlorine atoms in an equatorial position, and thus (a) while it lacks aromatic character, its molecular shape is more similar to that of strictly planar hexachlorobenzene than those of all other isomers and (b) it lacks any axial chlorine atom(s) which can be the site for 1,2-elimination by chemical mechanisms (Fig. 4).

This is one reason why this isomer shows a higher bioaccumulation (half-life in human body of about 7.2 years; Jung et al., 1997) and a higher bioaccumulation factor of 5274 (WHO, 1992). Since this isomer is eliminated very slowly from human body, it makes a significant contribution to the total HCH body burden. β-HCH was widely used on cotton plants during 1960s and 1970s.

β-HCH (1 μM) has mitogenic activity in MCF-7 cell lines in vitro (Coosen and van Velsen, 1989) but not in ERα−MDA-MB231 cell lines which express a non-functional ER (Steinmetz et al., 1996). This result indicates that a functional ER is necessary to elicit the mitogenic activity.

In vivo assay with mouse xenograft showed that 10−8 M β-HCH significantly increased MCF-7 cell number and at 10−5 M maximal responses were achieved (Steinmetz et al., 1996). Moreover β-HCH increased pS2 gene mRNA level. Like HCB, β-HCH does not compete with estradiol for binding to the ER (Coosen and van Velsen, 1989; Hatakeyama et al., 2002; Steinmetz et al., 1996). Its chemical structure characteristics may not allow binding the classical ligand binding domain and thus fail to activate ER in the nucleus, but it shows affinity to the alternative pocket and consequently triggers the Src/Ras/ERK pathway (Silva et al., 2010). Although unable to bind the ER, β-HCH is capable of activating ER target genes and the proliferation of estrogen-responsive cell lines (Silva et al., 2007; Steinmetz et al., 1996) by other chemical and biochemical mechanisms.

Treatment of MCF-7 cells with 1 × 10−5 M β-HCH resulted in strong activation of the Src kinase, ERK1 and ERK2. Thus β-HCH has been shown to induce rapid and sustained Src/ERK signaling in MCF-7 cell lines lasting for at least 30 min, while this pathway was not significantly affected by p,p′-DDE (Silva et al., 2010). Hatakeyama et al. (2002) observed similar activation of ERK1/ERK2 by β-HCH.

In epidemiological study Mussalo-Rauhamaa et al. (1999) found that β-HCH frequently occurs in breast fat of breast cancer patients with mean concentration of 0.13 ± 0.06 mg/kg fat. Controlling for age and parity, β-HCH remained a significant risk factor (OR, 10.51: 95% CI, 2.00–55.26). The cutoff point for the residue level in breast adipose tissue was >0.1 mg/kg fat.

2.2. Oxidative stress, apoptosis and OCPs’ toxicity

Apoptosis is the mechanism whereby damaged or unnecessary cells are naturally eliminated without causing damage to the surrounding tissue (Amiesen, 1996). This effect is accomplished by a partial, yet exhaustive digestion of cell structures (DNA, structural proteins, enzymes and lipids) prior to rupture of the cell membrane and release of products in the extracellular medium for clearance. When apoptosis is excessive or inappropriate, incomplete or insufficient, deflections trigger pathological-related condition such as immunodeficiency, autoimmune diseases, cancer (Alison and Sarraf, 1995; Gougeon et al., 1996) and reproductive anomalies, such as those due to abnormal spermatogenesis (Allan et al., 1992).

Oxidative stress, i.e., a shift of the cell redox balance towards a more oxidative (more negative) value of the electrochemical potential with respect to that of the relevant cell phase, is one main trigger in the induction of apoptosis (Kannan et al., 2000; Pérez-Maldonado et al., 2005). Many pesticides share as a possible mechanism of toxicity the ability to trigger apoptosis through alterations in redox homeostasis generated by a decrease of antioxidant defenses and by accumulation of reactive oxygen species (ROS). An over-production of ROS leads to processes such as oxidative modifications of redox signaling protein, oxidative DNA damage, endoplasmic reticulum stress and alterations in mitochondrial function which in turn trigger the activation of specific signaling cascades. Activation of stress-activated protein kinases (SAPKs) such as c-Jun N-terminal

Fig. 4. 3D molecular shapes of: (A) beta-hexachlorocyclohexane (β-HCH or 1,2,3,4,5,6-HCH [1α,2β,3α,4β,5α,6β]); (B) hexachlorobenzene (HCB) (Plots by ACD/Labs/3D, Advance Chemistry Development, 2007).
kinases (JNK) and of transcription-dependent p53 signaling cascades act as important sensors of electrophilic (and of oxidized) substances among which xenobiotic metabolites and induce apoptotic cell death. Pesticides also induce the activation of survival responses such as DNA repair mechanisms, mitogen-activated protein kinase/phosphatidylinositol-3-kinase (MAPK/PI3K) signaling cascades and up-regulation of antioxidant defenses in an attempt to cope with and counteract the deleterious effects of higher levels of intracellular reactive species which in turn trigger cell death pathways. In most cases apoptotic and survival signaling cascades are activated simultaneously in response to exposure to pesticides. Table 3 summarizes some studies on apoptotic effects of some OCPs.

DDT derivatives have been shown to induce neural cell death by apoptosis through the activation of mitogen-activated protein kinases (MAPKs) which play significant roles in controlling cell survival, proliferation, differentiation and cellular responses to various harmful signals (Shinomiya and Shinomiya, 2003). β-HCH, p,p′-DDE and other OCPs can induce elevation of ROS such as O₂⁻, HO* and NO (Samanta and Chainty, 1997; Song et al., 2008; Sujatha et al., 2001).

Shi et al. (2009) hypothesized the signaling pathways involved in p,p′-DDE-induced apoptosis: in his model ROS generation plays critical role in the initiation Sertoli cells apoptosis through two mechanisms. The first one is mitochondria-mediated pathway involving elevation of ROS, decrease in mitochondrial transmembrane potential along with the cytochrome c release from mitochondria into the cytosol and activation of the caspase-9 and -3. Other mechanism involves elevation of ROS, which resulted in activation of NF-κB, expression of Fasl and triggered FasL-dependent pathway (i.e. Fasl/caspase-8/-3 signaling module). In their investigation, Song et al. (2008) found at levels above 30 μM, p,p′-DDE induced apoptotic cell death of cultured rat Sertoli cells in a pro-oxidant and mitochondria dependent manner by activating the intrinsic programmed cell death pathway. Moreover p,p′-DDE elevated the apoptotic rate of Sertoli cells in vitro (at >30 μM) and germinal cells in vivo (at >20 mg/kg bw) by mechanism suspected to involve Fasl-dependent pathway (Shi et al., 2009, 2010).

Exposure to p,p′-DDE can enhance ROS production and oxidative stress, and then induce activation of NF-κB and expression of Fas-FasL. As a result, an intrinsic program of apoptotic death is stimulated in a target cell resulting to the activation of caspase-8. Ultimately, apoptosis of Sertoli cells and germinal cells is mediated by caspase 3, thereby disturbing the spermatogenesis (Shi et al., 2010). Song et al. (2011) reported the induction of Sertoli cell apoptosis by p,p′-DDE at above 30 μM through oxidative stress-mediated p38 MAPK and mitochondria-related pathway. Methoxychlor, which was intended to replace DDT, has been shown to induce testicular apoptosis in rats following oral exposure to single doses of 50 mg/kg bw where apoptosis was induced through involvement of Fas-FasL and mitochondria-dependent pathways (Vaithinathan et al., 2010). It is necessary to remark that the doses tested are unrealistically high if compared to those typical of occupational and environmental exposures.

Pérez-Maldonado et al. (2004) showed that o,p′-DDT, p,p′-DDT, p,p′-DDE and p,p′-DDT can induce apoptosis of human peripheral blood mononuclear cells in vitro at 20 μg/mL. This level is 400-fold higher than the mean blood total DDT level (0.05 μg/mL) observed in children in their pilot study (Pérez-Maldonado et al., 2004). The significant level of ROS, as detected by flow cytometry, was induced at 60 and 80 μg/mL (Pérez-Maldonado et al., 2005). A marginal influence of DDT and DDE on percentage of apoptosis was observed (p = 0.06) confirmed by a weak positive association between apoptosis and DDT exposure. In the follow-up study Pérez-Maldonado et al. (2006) found significant association between the percentage of apoptotic cells and the blood levels of only DDE of exposed children recruited in 2003 (p = 0.010) and those recruited in 2004 (p = 0.040). The association between DDT or DDE exposure and DNA damage was significant (p = 0.004 and p = 0.005 respectively), whereas the association between DDT or DDE and oxidative DNA damage and that of oxidative damage and apoptosis were not significant.

An isomer of DDD, o,p′-DDE, marketed under the name Mitotane is used as an antineoplastic medication in the treatment of adrenocortical carcinoma. Mitotane alters steroid peripheral metabolism, directly suppresses the adrenal cortex through the controlled destruction of adrenal tissue, which leads to a decrease in cortisol production (Maher et al., 1992; Wu et al., 2006).

Exposure of Wistar rats to a single dose of lindane (γ-HCH; 5 mg/kg bw) induced testicular apoptosis through the involvement of Fas-FasL and mitochondria-dependent pathways (Saradha et al., 2009). Shi et al. (2011) showed that concentrations >30 μM of β-HCH induced apoptotic cell death in rat Sertoli cells associated with increased expression of Fasl levels which could lead to the Fas activation. These two genes are known to induce apoptosis. Moreover β-HCH treatment induced an increase of nuclear factor kappa B (NF-κB) p65. The latter can directly stimulate the expression of these genes. Oxidative stress has been reported to enhance NF-κB activation (Nakamura and Omaye, 2008). β-HCH has been shown to induce activation of caspase-8, that plays the role in transduction of death signal (Said et al., 2004) and caspase-3, that initiates cell apoptosis (Khan et al., 2000).

Moreover, Shi et al. (2011) showed that β-HCH induces increase in apoptotic rate of Sertoli cells by possible mechanisms of ROS/JNK/Fasl pathway. In vitro exposure to β-HCH in rat Sertoli cells can enhance ROS and oxidative stress, and then induce activation of JNKs and NF-κB and expression of FasL. Significant increase of FasL protein expression was observed with 30–50 μM β-HCH treatment. This increase could lead to activate the Fas system. Upon engagement of Fas to Fas, an intrinsic program of apoptotic death is stimulated in a target cell leading to the activation of caspase-8. Finally, apoptosis of Sertoli cells is mediated by caspase-3, thereby disturbing the spermatogenic process (Shi et al., 2011; Fig. 5). These results led the authors hypothesize that ROS generation may play a critical role in the initiation of β-HCH-induced apoptosis by activation of the JNKs, translocation of NF-κB, expression of FasL and further activation of caspase cascade.

Yu et al. (2008) studied the individual effect of p,p′-DDE and β-HCH as well as the effect of their mixture on JNK and MAPK pathway in rat Sertoli cells. The latter were exposed to these OCPs at the final concentration of 10, 30 and 50 μM/L for 24h in each case. In both treatments, the expression of JNK and c-jun was elevated in a dose dependent manner after 24h of exposure. Liang et al. (2008) showed that at these levels p,p′-DDE, β-HCH and their mixture could induce apoptosis in rat Sertoli cells which was associated with activation of caspase-3 mediated by cleavage of caspase-8 and caspase-9. As stated for the case of p,p′-DDE above, these levels are also unrealistically high if compared even with the highest occupational exposures.

As for specific mechanisms, OCPs, which are themselves fairly un-reactive from the chemical point of view, are able to cause or trigger oxidative stress. However, little is known or hypothesized at the molecular level. Only the porphyrinogenic pesticide hexachlorobenzene is known for its ability to directly generate oxidative stress due to its unique property to act as an electron sink either as such or, more likely, through biotransformation products such as tetrachloro-1,4-benzoquinone (van Ommen and van Bladeren, 1989). That HCB is able to trigger apoptosis through the mitochondrial pathway has been demonstrated in the liver (Giribaldi et al., 2011) and in the thyroid (Chiappini et al., 2009).
2.3. Epigenetics and OCPs’ toxicity

Epigenetics is an emerging field of study of heritable changes that influence chromosomal stability and gene expression while not directly affecting changes in DNA sequence, such as by point mutations or chromosome disruption and re-assembly (Rodenhiser and Mann, 2006). One main mechanism of epigenetic modulation of genetic expression is methylation at 5-position of cytosines in repeated CpG sequences, a modification which suppresses expression of downstream DNA sequences. Thus suppression of specific genes or re-activation of silenced genes determines the destiny of individual cells towards quiescence or proliferation, thus opening the way to uncontrolled cell proliferation and cancer. Moreover, when epigenetic changes occur during certain stages of development, they become permanent and can be inherited by offsprings and disturbance of epigenetic modulation is thus thought to be an important mechanism in many diseases (Ozanne and Constancia, 2007).

Epigenetic effects have been hypothesized as a possible mechanism of POPs toxicity (Porta, 2006). For example, pregnant rats were treated with methoxychlor at the fairly high dose of 50–150 mg/kg during the sex-determining time window of pregnancy (days E7–E10). Gonads were collected from embryos in gestating mothers at E16 and from postnatal males showed a reduced germ cell to testis area at developmental age and an almost doubled fraction of apoptotic germ cells, although males were fertile and produced normal offspring (Cupp et al., 2003). In a further experiment (Anway et al., 2005) exposition of pregnant rats to 100 mg/kg produced male offsprings with reduced sperm capacity and fertility and the compromised fertility trait was passed through the adult male germ line for four generations. Altered patterns of DNA methylation was demonstrated to occur in the germ cells of generations two and three.

In vitro and animal studies have suggested that exposure to endocrine active compounds, such as POPs, may adversely affect DNA methylation patterns (Anway and Skinner, 2006; Anway et al., 2006). It has been proved that chemicals can alter the epigenetic marks and that epigenetic marks can be found in patients with the disease of concern or in diseased tissue (Baccarelli and Bollati, 2009).

DDT has been shown to affect DNA methylation in experimental animals as shown by Shuto et al. (2009). DDT was found to alter the methylation pattern in the hypothalamus of young rats (3 weeks of age) exposed at a dosage of 0.06 mg/kg/day. The 6 CpG islands were considerably hypomethylated as compared with controls. Thus the authors speculated that under low level of oxidation stress, the DNA methylation machinery malfunctions and this leads to incomplete methylation of specific gene regions. Following pyrosequencing methylation analysis of the rat livers from the rats exposed to high dose of mixture of twelve OCPs (1.9 mg/kg/day) showed no decrease in the methylation of CpG sites which is contrary to other tested chemical mixtures (Desaulniers et al., 2009).

To our knowledge only two epidemiological studies have been conducted to investigate exposure to POPs and DNA methylation levels in a human population. The first study was carried on Greenlandic Inuit and suggested a significant inverse linear relationship between DNA methylation and plasma concentrations of DDT, DDE, β-HCH, oxychlordane, α-chlordane and mirex (Rusiecki et al., 2008). This relationship was replicated by Kim et al. (2010) when they investigated the relationship between POPs exposure and DNA hypomethylation among healthy Koreans. These two studies demonstrated epigenetic changes related to environmental exposure although the two populations differ in magnitude of exposure, Greenland Inuit people being more exposed than Korean population.

3. Conclusions

The general population is exposed to OCPs through several ways, food being the major route of exposure. A few groups of the human population may show much higher levels of exposure than others due to the fact that they live in areas with a historical uncontrolled use of these pesticides in agriculture (typical examples being some areas of Central Asia) or that their diet mainly consists of bio-accumulating organisms, such as large marine mammals (the typical example is that of Arctic Inuits). In both cases the hazard is transmitted trans-generationally starting with breastfeeding of newborns and consequently the impairment of fertility can determine a demographic decline of some populations.

A number of adverse health effects such as endometriosis, infertility, immunotoxicity, neurotoxicity and spontaneous abortions, breast cancer, prostate cancer and neurodegenerative disorders have been suggested as a result of this exposure. OCPs may exert these effects through various mechanisms. Animal and in vitro
studies using cell cultures are most widely used to evaluate toxicity of these chemicals. OCPs act as agonists on ERα and/or antagonists on ERβ and also as probably antagonists on androgen receptors. These effects may contribute to the tumor promoting effects of these pesticides, which are observed in animals treated with high doses, which are not representative even of worst-case human exposure levels. Thus, p,p′-DDE and HCH have been shown to exhibit antiandrogenic effects by binding to ARs and competing with natural androgens, a characteristic which supports their estrogenic effect. Although HCB and β-HCH elicit estrogen-like responses, they do not compete with estradiol for binding to the ER like estrogen does. This suggests other intracellular pathways than the one used by endogenous estrogen in mediating these actions. They can elicit responses through AhR-dependent and independent pathways.

Many OCPs modulate or trigger apoptosis by redox signaling which involves alterations in antioxidant defenses and accumulation of ROS leading to oxidative stress. When not regulated, apoptosis can contribute to several diseases as immunodeficiency, autoimmune and cancer. However, limited information exists on full mechanistic events involved in the induction of cell death or survival by these pesticides.

Although not extensively explored, epigenetics has been considered to be a potential mechanism of POPs toxicity. Few studies have addressed OCPs and epigenetic modification as evident in human studies where only two studies have been conducted to elucidate the involvement of OCPs in health effects at epigenetic level. However some of these studies have shown OCPs may operate at epigenetic level. Thus more studies (both animals and human) are needed to document evidence of involvement of pesticides at epigenetics level in eliciting adverse health effects to human.

It is also important to remark that a very common characteristics of laboratory studies is that they have been carried out testing unrealistically high doses, and this makes the extrapolation to environmental and occupational human exposures very critical. However they produce hypotheses that deserve an investigation through specifically addressed epidemiological and experimental studies. This is particularly important because most of the epidemiological studies we have evaluated are not conclusive, and often results of different epidemiological studies are conflicting.

However, despite the not fully conclusive picture, it is evident that, having in mind the toxic potential, the bioaccumulation and biomagnification properties, there is a need to perform specific interventions addressed at reducing exposure, especially of vulnerable population subgroups. Thus stringent control in food safety system should be put in place to prevent the occurrence of OCPs in food and feedstuffs.

The restriction of the use and the ban of most of OCPs in most developed countries limits the possible health threats they might pose. Cessation of direct application on animals or presence of OCPs in their feed, housing or pasture will help to control them. Removal of the contaminated items from the human food chain can be opted when acceptable limits have been exceeded. Withdrawal times can be appropriate means for growing (meat-type) animals, which do not deliver a product on a daily basis. Finally, continuing research is needed on these contaminants, their body burden, potential health effects and ways to reduce their bioavailability in food. Moreover, effective policies are needed to control their manufacture and release into the environment.

Conflict of interest

The authors declare that there are no conflicts of interest.
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