MECHANISMS OF ACTIONS AND HEALTH EFFECTS OF ORGANOCHLORINE SUBSTANCES: A REVIEW.

Gourounti Kleanthi[1], Lykeridou Katerina[2], Protopapa Evaggelia[3], Lazaris Andreas[4]

1. MMedSc, MSc, RM, Laboratory Collaborator in Department of Midwifery, TEI of Athens
2. PhD, Associate Professor in Midwifery, Department of Midwifery, TEI of Athens
3. PhD, Professor in Aesthetics, Department of Aesthetics, TEI of Athens
4. PhD, Assistant Professor in Department of Anatomic Pathology, Medical School, University of Athens
.

Abstract

Organochlorines are a various group of synthetic chemicals that include polychlorinated biphenyls (PCBs), dibenzo- *p*- dioxiins and organochlorine pesticides. Human exposure to organochlorine substances may occur through inhalation of air, ingestion of food and water and skin absorption. Human exposure to organochlorines may occur not only during adulthood but also during prenatal and neonatal period. The developing fetus is exposed to organochlorines through placental transfer and the neonate through lactation. Organochlorine compounds exert many toxic effects on human health, such as, hormone related conditions (endometrisios, infertility), cancer of male and female reproductive system, developmental toxicity, neurotoxicity and immunotoxicity. The majority of these effects may be due to the ability of organochlorines to alter the levels of certain hormones, enzymes, growth factors and neurotransmitters and to induce key genes (cytochrome P-450 1A1 gene) involved in metabolism of steroids and xenobiotics. However, there is not always possible to identify causal relationships between organochlorine exposure and deleterious health effects. Limitations in the ability to identify or to quantify causal relationships are occasionally misinterpreted as evidence of safety. Frequently, governments have to wait until sufficient scientific information of harm is established before they act to prevent harm. However, failure to take precautionary action may have severe social, economic and health costs.

Key words: organochlorine substances, dioxins, endocrine disrupters, hormone related cancers, disorders of female reproductive system.

Corresponding Author:
Kleanthi Gourounti
123 Philellinon Keratsini 18756 Athens, Greece
Tel:6937673814.E-mail: clairegourounti@yahoo.gr

ISSN:1108-7366

Introduction

Over the last 60 years, a great number of man- made chemicals have been manufactured and many of them have become environmental pollutants (1). Many of these chemicals are organochlorine substances, which are organic compounds that contain chlorine (2). Many organochlorine compounds share several properties, such as: a) stability against decomposition or degradation by normal physical or biochemical processes because of their strong carbon-chloride bond, b) very low solubility in water, c) high solubility in hydrocarbon-like environment (lipophilicity), such as the lipid and fatty tissue. The properties of organochlorines made them very desirable for industrial use. As a result a class of 15.000 organochlorine chemicals approximately has been manufactured and widely used in past decades as industrial products (plasticizers, solvents, lubricants, dielectric fluids) and pesticides. Organochlorine substances are formed naturally in the environment, but only at low levels (3). Approximately 2000 compounds are known to be produced by living organisms. Organochlorines are a various group of synthetic chemicals that include polychlorinated biphenyls (PCBs), dibenzo- *p*- dioxins/ polychlorinated dibenzofurans (PCDDs/ PCDFs or dioxins) and organochlorine pesticides, such as dichlorodiphenyl-trichloroethane (DDT), lindane, aldrin and dieldrin (4,5). The most abundant of these man- made organochlorine compounds are the PCBs and the pesticide DDT, which were used widely in United States from 1945 (6).
The aim of this review was to present available information regarding the properties, mechanisms of actions and endocrine, genetic and carcinogenic effects of organochlorines on human health.

ORGANOCHLORINE SUBSTANCES AND THEIR PROPERTIES

Polychlorinated biphenyls (PCBs) are a group of 209 possible congeners with different numbers and positions of chlorine atoms on the aromatic rings (7). PCBs were first synthesized in the laboratory in 1881 and were started to be produced massively for commercial use in 1929. They have produced as technical mixtures (Aroclors, Phenoclors, Sovol, Clophens, etc) and have been used in industry as dielectric fluids in capacitors and transformers, plastisizers, flame retardants, lubricants and heat transfer fluids, and in the manufacture as paints and paper (4,7). PCBs are constituted as Persistent Organic Pollutants (POP), because of their chemical stability, their lipophilicity and consequently their ability to bioaccumulate in the environment (8). For this reason and for their potential harmful impact on humans and wildlife, their production and use was banned or severely restricted in United States, Canada and Western Europe since the late 1970s (9).

Dioxins (dibenzo-*p*-dioxins and polychlorinated dibensofurans) are also organochlorine substances that are produced as by-products (not produced deliberately) of a myriad of processes, including the incineration of garbage and hospital waste, the bleaching of pulp, the recycling of metals and the production of common solvents (2). Dioxin production seems inevitable whenever occurs combustion of organic matter in the presence of chlorine. Dioxins and furans are transported form place to place mainly in the atmosphere. Eventually they are deposited and bioaccumulate in plants and animals. The most toxic dioxin is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is assigned a value of Toxicity Equivalence Factor (TEQ) of 1.0.
DDT is a pesticide that first was used during World War II for control of lice and mosquitoes to combat typhus and malaria respectively (10). The World Health Organization has estimated that malaria-reduction programs by the use of DDT, have saved the lives of more than 5 million people (2). DDT was widely overused, particularly in agriculture and forestry, and consequently its environmental concentration rose rapidly and it began to affect the reproductive system of birds. Production and use of DDT peaked in the United States during the early 1960s (10)

pp:86-95
ISSN:1108-7366

and was banned by the Environmental Protection Agency (U.S.) in 1972 (11). During 1980s and 1990s many Third World countries banned the use of DDT in agriculture (10). The most prevelant breakdown product of DDT is the dichlorodiphenyldichloroethylene (DDE). DDE have similar properties with those of DDT.

ROUTES OF EXPOSURE

Human exposure to organochlorine substances may occur in many ways, including inhalation of air, ingestion of food and water and skin absorption (3). The major route of exposure to these substances is via food (and not drinking water) because of the bioaccumulation of organochlorines in fish and other animals that humans consume (12). It is estimated that more than 90% of PCBs and dioxins intake is through food (10). Because organochlorines are fat soluble, fish, meat and dairy products have the highest levels of them. Another route of exposure is through the long term and regular skin absorption of cosmetic products that contain organochlorines or other endocrine disrupters (13,14). The regular application of a variety of cosmetics with estrogenic activity to the underarm and upper breast area may lead to the continuous direct dermal exposure and consequently to the absorption and accumulation in underlying tissues (15).
Given that organochlorine compounds are lipid soluble and degrade slowly is expected to be bioaccumulated in human body and to be found in human adipose tissue, breast milk and blood. In general, the levels of organochlorine substances are about the same at different human tissues (adipose tissue, breast milk, muscle, blood). However, measurements in adipose tissue reflect steady the concentration of lipophilic chemicals while measurements in whole blood, serum and plasma may be influenced by blood lipids, as such, may be biased (5). Lately, human hair is used as a biologic measure of exposure to organochlorines and other persistent organic pollutants (16). Measurements of body burdens of organochlorine substances and their metabolites are good indicators of exposure and help to make associations between exposures and health outcomes (5).
Long term exposure to relatively small amount of organochlorines leads to the accumulation of these substances in human tissues. Therefore, levels of organochlorines in human tissues are positively associated with age (17) and with rate of consumption of polluted products (2). It has been observed that vegetarians (i.e. eat all vegetables, fruits and grain with no animal products) have much lower levels of organochlorines compared to individuals who consume animal- based products (2). Additionally, inhabitants of developed countries (North America and Western Europe) have lower levels of organochlorines than inhabitants of developing countries, probably reflecting differences in exposure (4).

PRENATAL AND ANTENATAL EXPOSURE TO ORGANOCHLORINES

Human exposure to organochlorine substances may occur not only during adulthood but also during prenatal and neonatal period. The developing fetus is exposed to organochlorine substances and other persistent organic pollutants through placental transfer (18). Placenta cannot prevent the entrance of organochlorines in the embryonic circulation. The *in utero* exposure to these substances (PCBs, DDT, DDE and dioxins) is determined by analyzing the blood of infants' umbilical cord (2). However, a recent study suggests that placenta may restrict the transfer of organochlorines from maternal to fetal circulation (19). This study showed that organochlorines accumulate in the placenta and their concentration was greater in maternal blood than cord blood. Exposure to organochlorine compounds continues postnatally via lactation. Since organochlorines are lipophilic, they are excreted in breast milk. Therefore, human breast milk is the major source of exposure of newborn infants. The postnatal exposure of children is assessed by analyzing their mother's breast milk and also by analyzing children's blood at the age of four years (2). It is well established that organochlorines

are mobilized from fat stores only during periods of starvation or lactation (20). Many studies have shown that the levels of these substances decline significantly during the energetically expensive period of pregnancy and lactation (21,22). Lactation is the major route by which women excrete organochlorines (23). Thus, it could be concluded that infant has already a burden of organochlorine substances from the first months of its life (24). Because the fetal and the neonatal period are important periods regarding the development and differentiation, the possibility of exposure to organochlorines during this time is particularly of concern.

There is evidence that suggests that mammals are more susceptible to organochlorines and other persistent organic pollutants during fetal and neonatal period than adulthood (22). There are many reasons why fetus and neonate are more sensitive to exposure to organochlorines. Firstly, the fetus may be exposed to these substances during the very sensitive and crucial period of organogenesis and development. Secondly, because many of the normal detoxification mechanisms and the immune system of the fetus and of the infants are not fully developed, exposure to low doses of organochlorines may have adverse effects (25). Thirdly, fetuses and infants exposed to unusually high levels of organochlorines for their total body mass that cannot metabolize or excrete (20).

HEALTH EFFECTS OF ORGANOCHLORINES

There is a growing concern lately that organochlorine compounds exert many toxic effects on health of human and wildlife populations (26,27). The toxic effects observed in human include disorders in the female and male reproductive system and infertility, carcinogenicity, developmental toxicity, neurotoxicity and immunotoxicity (28,29,30). Dioxins and particularly TCDD is a known human carcinogen (31). The majority of these effects may be due to the ability of organochlorines to alter the levels of certain hormones, enzymes, growth factors and neurotransmitters.

ENDOCRINE DISRUPTING EFFECT OF ORGANOCHLORINES

Organochlorines can impact on human health by disturbing the balance of endocrine system and therefore are known as hormone disrupting chemicals or as endocrine disrupters (3). More general names of these substances are environmental hormones, synthetic hormonally active agents (HAAs) and xenoestrogens. The hypothesis that many environmental pollutants have hormonal action is not new. In recent years, attention has focused on the potential of some chemical to act as endocrine disrupters (4). According to the (U.S.) Environmental Protection Agency an endocrine disrupter is defined as “a chemical that interferes with the function of the endocrine system by mimicking a hormone, blocking the effects of a hormone, or by stimulating or inhibiting the production or transport of hormones” (31). Chemicals that act as endocrine disrupters alter the levels of hormones and particularly the levels of steroid hormones. Hormone disrupters may disturb the endocrine system by various ways. These chemicals bind to specific hormone receptors and thereby ‘mimic’ or block the attachment of endogenous hormone to its receptor (32,33). Endogenous hormones (estradiol) and organochlorine chemicals with endocrine disrupting action (estrogenic action) contain the characteristic four-ring steroid structure.

However, most of these compounds bind to the receptor with only a small fraction of strength of endogenous hormones (estrogens). Therefore, many organochlorines and/ or their metabolites have shown weak estrogenic effects in vitro and in some in vivo experiments (35). It has been found that steroidal estrogens are far more potent than estrogen-like chemicals (such as DDT) (34,35). It is estimated that approximately 10.000 fold higher doses of DDT than 17β estradiol are required to produce comparable effects in assays (33). However, it has been found that multiple estrogenic chemicals can act in combination and consequently produce an effect even when are present at low levels which on

their own would not induce an observable effect (35,36). Endocrine disrupters may also act indirectly. They may affect the pattern of synthesis and production of endogenous hormones, alter their structure and their metabolism via induction of enzymes, change the number of hormone receptors and effect on estrogen responsive genes (37). PCB congeners, according to their different pattern of chlorination, have the ability to induce enzymes that are involved in the metabolism of different hormones (7).

Many organochlorine compounds, such as DDT, PCBs and dioxins are considered as endocrine disrupters because they are weakly estrogenic or anti-estrogenic in experimental assays (32). Organochlorines have been shown to affect not only steroids hormones but also thyroid hormones. It has been revealed that dioxins and particular TCDD has anti-estrogenic properties. Dioxins exert their biological effect via binding not only to steroid receptors but also to a specific receptor, the aryl hydrocarbon (Ah) receptor (38). Some PCBs have estrogenic effects, while some others have structural similarities with dioxins, bind with Ah receptor and consequently have anti-estrogenic effects (34). According to Wolff and Toniolo (39), PCBs congeners can be classified into three groups, on the basis of their structural and biological properties. Group I includes congeners that are potentially estrogenic (*ortho*-congeners). Group II includes congeners (mono-*ortho* or non-*ortho*) that have structural similarities with dioxins and are potentially anti-estrogenic. Group III includes congeners that are phenobarbital-type.

Up till now, more than 500 chemicals have found to be weakly estrogenic, including many common chemicals, such as pesticides and plastics (40,41). Some of these compounds are suspected to disrupt the endocrine system by mimicking estrogenic activities and thereby increase the risk of hormone dependent disorders such as endometriosis, early menarche and male and female infertility.

ORGANOCHLORINES AND GENE INTERACTION

Organochlorines and particularly PCBs are strong inducers of key genes involved in metabolism of steroids and xenobiotics, such as cytochrome P-450 1A1 (CYP1A1) gene (42). CYP1A1 is a gene that involves in metabolism of steroids and many potentially genotoxic chemicals (43). In humans, CYP1A1 is under the regulatory control of the aryl hydrocarbon (Ah) receptor, which is a transcription factor that regulates gene expression (44). CYP1A1 gene encodes cytochrome P-450 1A1 enzyme, which is a key enzyme in phase I of bioactivation of xenobiotics (45). CYP1A1 also encodes the aryl hydrocarbon hydrolase (AHH) (46). Aryl hydrocarbon hydrolase is an enzyme which is involved in the metabolism of steroid hormones, polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds. Cytochrome P-450 1A1 contributes to the activity of the aryl hydrocarbon hydrolase by catalyzing the first step in the metabolism. Cytochrome P-450 1A1 is also involved in estrogen metabolism, catalyzing the hydroxylation of 17β estradiol (44).

As it was referred above, dioxins and some PCBs congeners (non-ortho congeners) bind to the Ah receptor. The proposed mechanism of reaction of organochlorines with DNA involves the **cy**tochrome **P**4501A1 (CYP1A1) gene (47). The binding of organochlorines with Ah receptors seems to trigger the expression of gene CYP1A1 which encodes the enzymes that are involved in the metabolism of organochlorines. Metabolism of organochlorines within adipose tissue can generate reactive oxygen intermediates that cause oxidative damage to DNA and consequently cause DNA mutation (48). These latter substances are known to be carcinogenic. Therefore, organochlorines have the ability to induce enzymes that are involved in the metabolism of several chemical carcinogens and hormones, such as estradiol (39).

According to this mechanism, individuals with higher activity of CYP1A1 gene would be at greater risk of cancer (such as breast cancer) when exposed to higher levels of organochlorines (48). Four polymorphisms have been identified in CYP1A1 gene (48). Polymorphisms of CYP1A1 include M1 (T→C

substitution at nucleotide 3801), M2 (A→G substitution at nucleotide 2455 leading to an amino acid change of isoleucine to valine at codon 462), M3 (T→C substitution at nucleotide 3205) and M4 (C→A substitution at nucleotide 2453 leading to an amino acid change of threonine to asparagines at codon 461) (48). Recent studies have managed to associate a specific polymorphism (M2) of the *CYP1A1*gene with higher interaction with PCBs and consequently with higher production of enzymes. Thus, high accumulation of PCBs in adipose tissues could induce the production of AHH, through interaction with *CYP1A1* or the polymorphic *CYP1A1 M2* allele, and high amounts of carcinogenic intermediates could be produced.

CARCINOGENIC EFFECT OF ORGANOCHLORINES

Historically, carcinogenesis has been characterized by three separate stages: initiation, promotion and progression (26). Initiation is the first stage of carcinogenesis and represents the occurrence of an irreversible change in a cell. This change is probably a genetic change or a mutation that results in a neoplastic cell and is caused by an initiating agent (e.g. chemical substance) that causes damage to DNA. Promotion is the second stage of carcinogenesis and is characterized by a clonic expansion of the initiated (mutated) cell to a benign tumor. The clonic expansion of initiated cells is not autonomous and is dependent on the repeated exposure to the promoter. Promoter may be an exogenous or an endogenous chemical substance, such as a hormone. Progression is the third stage of carcinogenesis and is characterized by the autonomous clonic expansion of mutated cells, even when the promoter is not present. Progression describes the irreversible transition from a benign to a malignant tumor. Chemicals that act as initiators and directly damage DNA are known as genotoxic. Chemicals that act as promoters are not able to produce mutations on their own but can increase the risk of cancer by increasing and stimulating the growth rate of mutated cells. Carcinogenic agents are often genotoxic, or able to damage DNA (26). Initiation involves genotoxicity, whereas promotion involves stimulation of cell proliferation.

The International Agency for Research on Cancer (49) has categorized agents, mixtures and exposures to five categories: carcinogenic to humans (class 1), probably carcinogens to humans (class 2A), possibly carcinogenic to humans (class 2B), not classifiable as to carcinogenicity in humans (class 3), probably not carcinogenic to humans (class 4). IARC has already evaluated 885 agents including several organochlorine compounds (49). The potential carcinogenic risk to humans of organochlorine compounds which may act as endocrine disrupters has been covered in great detail by IARC. The overall conclusions drawn were that TCDD is human carcinogen (class 1), PCBs are possibly carcinogenic to humans (class 2A) and DDT is probably carcinogenic to human (class 2B). The first class includes substances for which human studies (epidemiological or/and experimental studies) provide sufficient evidence of carcinogenicity in humans. The second class includes substances for which there is limited evidence about their carcinogenicity in human or/ and sufficient evidence of carcinogenicity in experimental animals. The Environmental Protection Agency (EPA) uses a very similar classification scheme to IARC scheme. EPA (50) classified both PCBs and DDT as probable human carcinogens (Category 2B).

There is much evidence from epidemiological studies that suggests that PCBs are complete carcinogens that act as initiators (possibly through an oxidative stress mechanism) and as promoters (probably involving the Ah receptors) (51). Animal studies using high doses of PCBs suggest that PCBs are both tumorigenic and carcinogenic (51).

The concern that environmental organochlorine pollutants may cause cancer in humans is widespread (52). Evidence for the potential carcinogenic effects of chemicals that act as endocrine disrupters comes from the 'Diethylstilbestrol (DES) syndrome'. Fetal exposure to this drug

resulted in abnormalities of male and female reproductive tract and a large increase of vaginal adenocarcinoma in young women (53).

A great increase in the incidence of cancer has been observed the last years, especially for hormonally related cancers, such as breast, endometrium, prostate and testis cancer (54). Hormones play a major role in the etiology of several human cancers, including cancer of the breast, endometrium, ovary, prostate, testis and thyroid (55). Some researchers have noted that endometrial cancer is a natural focus to study estrogenic activity-cancer related to organochlorines (56) and that estrogenic effects of certain organochlorines would be easier to be detected on the endometrium. Therefore, lately there is a growing concern about the association between organochlorines and the risk of endometrial cancer. However, the studies that have investigated the association between endometrial cancer and levels of organochlorines and other endocrine disrupters are few. In general, these studies suggested that there is no association between endometrial cancer and exposure to organochlorines (52,57).

Conclusion

In conclusion, the evidence to date concerning the health effects of organochlorines suggests that organochlorine compounds may exert toxic effects on human health through various mechanisms of action. Exposure to organochlorine chemicals has been associated with many deleterious effects on human health, such as, hormone related conditions (endometrisios, infertility), cancer of male and female reproductive system, immunotoxicity, neurotoxicity and spontaneous abortions. However, there is not always possible to identify causal relationships between organochlorine exposure and deleterious health effects. Limitations in the ability to identify or to quantify causal relationships are occasionally misinterpreted as evidence of safety. Therefore, when present activities entail potential, unknown adverse health effects, the need for more accurate evidence has often been used as a reason for inaction. Frequently, governments have to wait until sufficient scientific information of harm is established before they act to prevent harm. Failure to take precautionary action may have severe social, economic and health costs. The *precautionary principle* states that, in cases of serious or irreversible threats to the health of humans or ecosystems, acknowledged scientific uncertainty should not be used as a reason to postpone preventive measures. These preventive precautionary actions have the aim to reduce and if possible to remove exposures to potentially harmful substances, activities and other conditions. The precautionary principle requires that chemicals should not be discharged into the environment until they are proven to be harmless. This is the opposite to the usual process of risk assessment, which consider that chemicals are safe and harmless until proven the harmless and dangerous. Therefore, the implementation of precautionary principle avoids difficulties that may arise from limitations of assessing the toxic effects of chemicals on health. The precautionary actions that should be taken in case of organochlorine substances are :

- Replacement of organochlorine substances with less dangerous alternative substances.
- Re-evaluation of production processes, products and human activities.
- Provision of information and education to the public in order to minimize the exposure to possibly harmful substances, such as organochlorines.

Bibliography

1. Longanathan B. and Kannan K. Global organochlorine contamination trends: an overview. *AMBIO* 1994;23: 187-189
2. Baird C. and Cann M. *Environmental Chemistry*, 3rd edition. New York, W.H. Freeman and Company, 2004.
3. Nicolopoulou- Stamati P. and Pitsos M. The impact of endocrine disrupters on the

female reproductive system. *Human reproduction Update*. 2001;7(3): 323-330
4. Calle E., Frumkin H., Henley J., Svitz D., Thum M. Organochlorines and breast cancer risk. *Cancer J Clinicians*, 2002;52: 301-309
5. Petreas M., Smith D., Hurley S., Jeffrey S., Gilliss D., Reynolds P. Distribution of persistent, lipid soluble chemicals in breast and abdominal adipose tissues: lessons learned from a breast cancer study. *Cancer Epidemiol Biomarkers Prev*, 2004;3(3): 416-424
6. Kutz FW, Wood PH, Bottimore DP. Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. *Rev Environ Contam Toxicol* 1991;20:1-82.
7. Negri E., Bosetti C., Fattore E., La Vecchia C.. Environmental exposure to polychlorinated biphenyls (PCBs) and breast cancer: a systematic review of the epidemiological evidence. *Eur J Cancer Prev*. 2003 ; 12(6): 509-16
8. Charlier C., Albert A., Zhang L., Dubois N., Plomteux G. Polychlorinated biphenyls contamination in women with breast cancer. *Clinica Climica Acta*, 2004;347: 177-181
9. WHO (1993). Polychlorinated Biphenyls and Terphenyls, 2nd edn. Geneva, Switzerland: Environmental Health Criteria 140. *World Health Organization*.
10. Snedeker S. Pesticides and breast cancer: a review of DDT, DDE and dieldrin. *Environmental Health Perspectives*, 2001;109(1): 35-47
11. US EPA. (1990) Suspended, cancelled and restricted pesticides, EPA 20T-1002. Washington, DC: US. Environmental Protection Agency
12. Hall R. A new treat to public health: organochlorines and food. *Nutr. Health*, 1992;8: 33-43
13. Darbre P. Environmental estrogens, cosmetics and breast cancer. *Best Practice and Research Clinical Endocrinology and Metabolism,* 2006; 20(1): 121-143
14. Donovan M., Tiwary C., Axelrod D., Sasco A., Jones L., Hajek R. et al (2006) Personal care products that contain estrogens or xenoestrogens may increase breast cancer risk. *Medical Hypotheses* (article in press).
15. Harvey P. and Darbre P. Endocrine disrupters and human health: could estrogenic chemicals in bodycare cosmetics adversely affect breast cancer incidence in women? A review of evidence and call for further research. *Journal of Applied Toxicology.* 2004;4: 167-176
16. Althuis M., Dozier J., Anderson W., Devesa D., Brinton L. Global trends in breast cancer incidence and mortality 1973-1997. *Int J Epidemiol* 2005;34 (2): 405-12
17. Cocco P. On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects. *Cad Saude Publica*, 2002;18(2): 379-402
18. Ando M., Saito H., Wakisaka I. Gas chromatographic and mass spectrometric analysis of polychlorinated biphenyls in human placenta and cord blood. *Env Res* 1986;41: 14-22
19. Suzuki G., Nakano M., Nakano S. Distribution of PCDDs/PCDFs and Co-PCBs in human maternal blood, cord blood, placenta, milk and adipose tissue: dioxins showing high toxic equivalency factor accumulate in the placenta. *Biosci Biotechnol Biochem* 2005;69(10): 1836-1847.
20. Thomas K. and Colborn T. (1992) Oganochlorine endocrine disruptors in human tissue. In: Colborn T. and Clement C., eds. *Chemically-induced alterations in sexual and functional development: the wildlife/human connection*. Princeton Scientific Publishing Co, INC Princeton, New Jersey.
21. Dewailly E., Ayotte P., Laliberte C., Weber P., Gingras S. and Nantel A. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethylene (DDE) concentrations in the breast milk of women in Quebec. *Am J Public Health*, 1996;86: 1241-1246
22. Sweeney T. Is exposure to endocrine disrupting compounds during fetal/post-natal development affecting the

reproductive potential of farm animals? *Domestic Animal Endocrinology*, 2002;23: 203-209
23. Romieu I., Hernandez-Avila M., Lazcano-Ponce E., Weber J., Dewaily E. Brest cancer, lactation history, and serum organochlorines. *American Journal of Epidemiology*, 2000;152(4): 363-370
24. Patandin S., Dagnelie P., Mulder P., Op de Coul E., van der Veen J.E., Weisglas-Kuperus N., et al. Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: a comparison between breast feeding, toddler and long term exposure. *Environmental Health Perspectives*,1999; 107: 45-51
25. Crisp T., Clegg E., Cooper R., Wood W., Anderson D., Baetcke K., et al. Environmental endocrine disruption: an effects assessment and analysis. *Res Environm Health*, 1998; 106: 11-56
26. Brody J. and Rudel R. Environmental pollutants and breast cancer. *Environmental Health Perspectives* 2003;111(8): 1007-1019
27. Safe S. Endocrine disruptors and human health: is there a problem. *Toxicology*, 2004;205: 3-10
28. Skakkebaek N., Rajpert-De M., Main K. Testicular dysgenesis syndrome : an increasingly common developmental disorder with environmental aspects. *Hum. Reproduc.2001;* 16: 972-978
29. Teilmann G., Juul A., Skakkebaek N., Toppari J. Putative effects of endocrine disrupters on pubertal development in the human. *Best Practice and Research Clinical Endocrinology and Metabolism*, 2002;16(1): 105-121
30. Toft G., Axmon A., Giwercman A., Thulstrup A.M., Rignell-Hydbom A., Pedersen H.S., et al. Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross sectional study. *Environmental Health: A Global Access Science Source*, 2005; 4: 26-37
31. EPA (US Environmental Protection Agency). (2001) Dioxin Reassessment. [available: http://cfpub.epa.gov/ncea/cfm/dioxin].
32. Soto A., Sonnenscjein C., Chung K., Fernandez M., Olea N., Serrano F. The E-SCREEN assay a tool to identify estrogens: an update an estrogenic environmental pollutants. *Envirom Health Perspect*. 1995;103: 113-122
33. Shelby M., Newbold R., Tully D., Chae K., Davis V. Assessing environmental chemicals for eastrogenicity using combination of in vitro and in vivo assays. . *Envirom Health Perspect*. 1996;104: 1296-1300
34. Safe SH Environmental and dietary estrogens and human health: is there a problem? *Environ Health Perspect* 1995;103: 346–351.
35. Kortenkamp A. Breast cancer, oestrogens and environmental pollutants: a re-evaluation from a mixture prospective. *International Journal of Andrology,* 2006; 29(1): 193-8.
36. Silva, E., Rajapakse, N., Kortenkamp, ASomething from nothing – eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environmental Science & Technology*. 2002;36, 1751–1756.
37. NRC (National Research Counsil) (1999) *Hormonally active agents in the evironment*. Washington, DC: National Academy Press.
38. Birnbaum L., and Fenton S. Cancer and developmental exposure to endocrine disruptors. *Environmental Health Perspectives*, 2003;111(4): 389-394
39. Wolff M., Toniolo P., Lee E., Rivera M., Dublin N. Blood levels of organochlorine residues and risk of breast cancer. *JNCI* 1993;85: 648
40. Jobling S., Reynolds T., White R., Parker M., Sumpter J. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environmental Health Perspectives.* 1995;103: 582-587
41. Nishihara T., Nishikawa J., Kanayama T., Dakeyama F., Saito K., Imagawa M., et al. Estrogenic activities of 517 chemicals by yeast two-hydric assay. *J Health Science* 2000;46: 282-298
42. Safe S. Polychlorinated biphenyls (PCBs): environmental impact,

biochemical and toxix responses, and implications for risk assessment. *Crit Rev Toxicol*, 1994; 24; 87-149

43. Zhang Y., Wise J., Holford T., Xie H., Boyle P., Hoar S., et al Serum polychlorinated biphenyls, cytochrome P-450 1A1 polymorphisms, and risk of breast cancer in Connecticut women. *American Journal of Epidemiology*. 2004;160(12): 1177-1183
44. Masson L., Sharp S., Cotton S., Little J. Cytochome P-450 gene polymorphisms and risk of breast cancer: a huge review. *American Journal of Epidemiology*, 2005; 161(10): 901-915.
45. Nebert D. Role of genetics and drug metabolism in human cancer risk. *Murat Res*, 1991;247: 267-81.
46. Whitlock J.Induction of cytochrome P450A1. *Ann. Rev. Pharmacology Toxicology*.1999;39: 103-125
47. Bandiara S., Torok S., Latcher R., Norstrom R. Immunoquantitation of cytochrome P450 1A and P450 2B and comparison with chlorinated hydrocarbons levels in archived polar beat liver samples. *Chemosphere*, 1997; 34: 1469- 1479
48. Li Y., Millikan R., Bell D., Cui L., Tse C., Savitz D., Beach J., Edmiston S., et al. Dichlorodiphenyldichloroethene, polychlorinated biphenyls, and breast cancer among African- American and white women in North Carolina. *Cancer Epidemiol Biomarkers Prev*, 2004; 9: 1233-1240
49. International Agency for Research on Cancer (IARC), (2003) IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Human [available at http://www.iarc.fr, last updated: 9 January 2003].
50. EPA (US Environmental Protection Agency). (1998) DDE p,p Dichlorodiphenyldichloroethylene, Washington DC: Office of Pesticide Program and Toxic Substances.
51. Faroon O., Keith S., Jones D., DeRosa C. Carcinogenic effects of polychlorinated biphenyls. *Toxicology and Industrial Health*, 2001;17: 41-62
52. Weiderpass E., Adami HO., Baron J., Wicklund- Glynn A., Aune M., Atuma S., Persson I. Organochlorines and endometrial cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, 2000;9: 487-493.
53. Henderson B., Benton B., Cosgrove M., Baptista M., Aldrich J., Townsend D., Hart W., Mack T. Urogenital tract abnormalities in sons of women treated with diethylstilbestrol. *Pediatrics*, 1976;58: 505-507
54. Charlier C., Albert A., Herman P., Hamoir E., Gaspard E., Meurisse M. et al. Breast cancer and serum organochlorine residues. *Occup Environ Med*, 2003; 60: 348-351
55. Cocco P. On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects. *Cad Saude Publica*, 2002;8(2): 379-402
56. Garcia A. Pesticide exposure and women's health. *American Journal of Industrial Medicine*, 2003;4: 584-594
57. Sturgeon S., Brock J., Potischman N., Needham L., Rothman N., et al. Serum organochlorine compounds ad endometrial cancer risk (United States). *Cancer Causes Control*, 1998;9: 417-424.