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Τμήμα Χημείας, Πανεπιστήμιο Αθηνών
ΕΠΙΣΤΗΜΟΝΙΚΑ ΘΕΜΑΤΑ

Περιβαλλοντική Ρύπανση από Χημικούς Ενδοκρινικούς Διαταράκτες. Επιπτώσεις στην Ανάπτυξη, Αναπαραγωγή και Ανοσολογικό Σύστημα των Ζώων Άγριας Φύσης και στην Υγεία του Ανθρώπου

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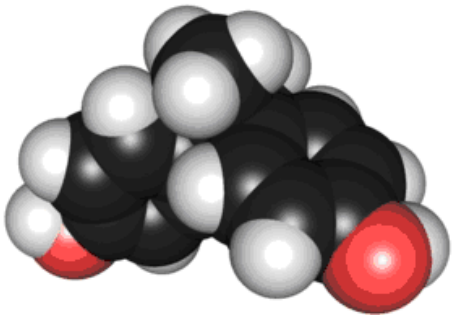

Environmental Pollution by Endocrine Disrupting Chemicals. Adverse Developmental, Reproductive and Immune Effects in the Wildlife and in Human Health

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Περίληψη

Οι ενδοκρινικοί διαταράκτες είναι φυσικές ή συνθετικές χημικές ουσίες οι οποίες παρεμβαίνουν στην ομαλή λειτουργία του ορμονικού συστήματος τόσο του ανθρώπου, όσο και των ζώων. Πολλές χημικές ουσίες, φυτοφάρμακα, διάφορα καταναλωτικά προϊόντα και αλογονωμένοι ρύποι είναι αρκετά διαδεομένοι στο περιβάλλον. Η έκθεση στους ενδοκρινικούς διαταράκτες μπορεί να συμβεί από την τροφή, το νερό, τον αέρα και το έδαφος, αλλά και επιπρόσθετα κατά την εμβρυϊκή φάση. Μέχρι τώρα έχουν προσδιορισθεί πολλές φυσικές και συνθετικές ουσίες, οι οποίες επιδρούν στη θυρεοειδική λειτουργία ζώων και ανθρώπου με πολλαπλούς μηχανισμούς: Η έκθεση στους ενδοκρινικούς διαταράκτες αφορά και την υγεία του ανθρώπου. Κυρίως είναι περισσότερο ευάλωτος στις χημικές αυτές ουσίες κατά την εμβρυϊκή ζωή και κατά την παιδική ηλικία, καθώς μπορεί να επέμβουν σε διάφορα στάδια της ανάπτυξης. Υπάρχει πάντοτε ένα σημαντικό χρονικό διάστημα από την έκθεση στους ενδοκρινικούς διαταράκτες έως την εμφάνιση των συμπτωμάτων, γεγονός που καθιστά τη μελέτη της δράσης τους ιδιαίτερα δύσκολη. Πολλές έρευνες έχουν πραγματοποιηθεί με θυρεοειδικοί διαταράκτες και έχουν μελετηθεί αποκλειστικά *in vitro* ή σε πειραματόζωα, ενώ η δράση τους στον άνθρωπο παραμένει υπό διερεύνηση. Στην ανασκόπηση αυτή περιλαμβάνονται σημαντικές ερευνητικές εργασίες, περιβαλλοντικές αναλύσεις και επιδημιολογικά δεδομένα για ενδοκρινικούς διαταράκτες σε ζώα της άγριας φύσης και στον άνθρωπο. Συγχρόνως γίνεται συστηματική αναφορά σε μεγάλο αριθμό επιστημονικών δεδομένων και εκθέσεων διεθνών οργανισμών για την πρόοδο που έχει σημειωθεί στην διερεύνηση των μηχανισμών και εκτιμήσεις κινδύνου για μεγάλο αριθμό χημικών ουσιών και παρασκευασμάτων. Η ανασκόπηση ολοκληρώνεται με επίκαιρες και πρόσφατες μελέτες για το επιστημονικό στάδιο αντιμετώπισης των αρνητικών συνεπειών στο ανθρώπινο περιβάλλον.

Abstract

Endocrine disrupters (EDCs) are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. Natural and man-made chemicals were tested for ED effects in biological organisms. EDC include dioxin,

polychlorinated biphenyls, DDT and other polychlorinated pesticides, plasticizers (such as bisphenol A), phthalates and a variety of other synthetic organic chemicals. The hypothesis that hormonally active compounds in the environment are having a significant impact on human and ecological health has captured the public's attention like no other toxicity concern since the publication of Rachel Carson's *Silent Spring* in 1962 (about DDT). In the early 1990s, T. Colborn and other scientists began to collect scientific data and environmental information about the potential impacts of endocrine-mediated toxicity in the environment and human health. The EDCs issue has been for more than twenty years and it is now on the agenda of many expert groups of governmental organizations, industry and academia in industrialized countries. EDCs pollution generated considerable concerns for human health. But, after substantial and vigorous analysis of human data has so far failed to provide firm evidence of direct causal associations between low-level (i.e., levels measured in the general population) exposure to chemicals with EDCs and adverse health outcomes in humans. Some scientists suggest that low concentrations can have detrimental effects in the first stages of development. In the last twenty years several field and laboratory studies have shown that exposure in polluted aquatic environments to certain EDCs has contributed to adverse effects in some wildlife species and populations. These effects vary from subtle changes in the physiology and sexual behaviour of species to permanently altered sexual differentiation. In this review we present the most recent and important research papers and reports on EDCs in the environment and their impact of wildlife, terrestrial and aquatic biota, and secondly reviews and papers on the results of the human data from exposure to chemical with EDCs and adverse health outcome to humans.

1. Introduction : Endocrine Disrupting Chemicals

For a number of years, concern has been growing over the health effects of chemical pollutants associated with disruption of hormonal systems in living organisms, especially wildlife, and humans. In the beginning of 1990s the issue of endocrine disrupters (or disruptors, EDCs) became the focus of considerable media attention throughout the world [1].

The public's attention and the perception associated with health threats from pesticides was already very strong since the publication of Rachel Carson's "Silent Spring" in 1962 and the DDT story in the USA [2]. In the early 1990s, Theo Colborn (she is Professor Emeritus of Zoology at the University of Florida, Gainesville) and other scientists started collecting scientific papers and research projects about the

potential impacts of endocrine-mediated toxicity in the wildlife and humans [3, 4]. The book in the popular press with the impressive title "*Our Stolen Future: Are We Threatening our Fertility, Intelligence and Survival? A Scientific Detection Story*" (1997) had a great impact on the American public. Alerted many scientists for a widespread and "emerging" environmental pollution and possibly an important human health threat [5].

In the last decades many of the polychlorinated chemicals , pesticides, polybrominated aromatic compounds and many of their applications have been restricted of banned completely. The initial epidemiologic studies that were positive for adverse health effects from EDC exposure were reverses or their risk decreased substantially. But there is undeniably some damage to health, especially for young infants and in the wildlife from exposures and environmental pollution in general from EDC pollution. Phytoestrogens in our diet and hormonal changes in humans are influencing the adverse effects on humans. In the case of EDC pollution and reproductive effects in wildlife species, dramatic changes occur only in special cases of high and prolonged pollution by toxic waste and pesticides.

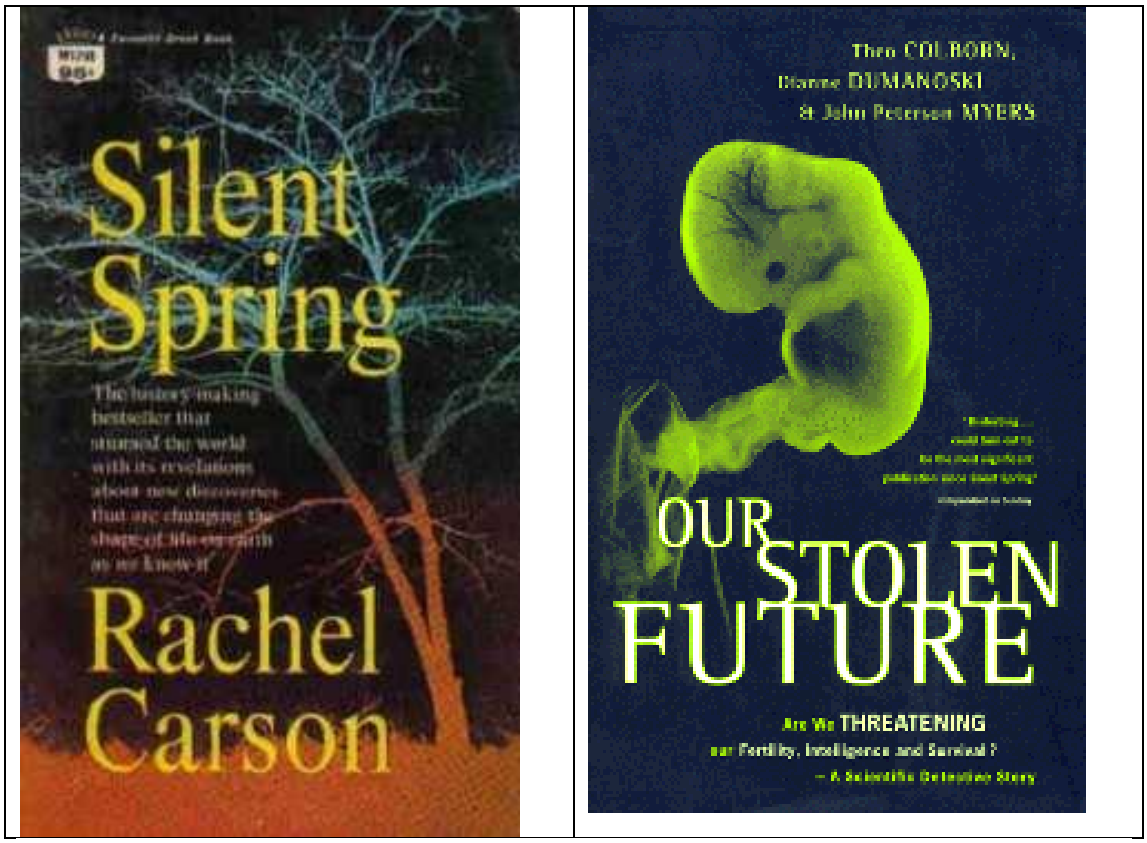


Figure 1. In the 1990s there were many publications on endocrine disrupters. Rachel Carson "Silent Spring" (1962). Colborn T et al. "Our Stolen Future" (1997)

2. Research Efforts on Endocrine Disrupting Chemicals in the USA

Recognizing the possibility that EDCs of an emerging health threat, the U.S. Environmental Protection Agency (EPA) convened two international workshops in 1995 that identified research needs relative to future risk assessments for endocrine-disrupting chemicals (EDCs). These workshops identified potential adverse effects from extensive exposure on reproductive, neurological, and immunological function, as well as carcinogenesis as the major endpoints of concern and made a number of recommendations for future research. [6, 7]

In the USA, the issue of EDCs in the 1990s started to become a serious environmental and health issue. Subsequently, the EPA developed a research strategy to begin addressing the recommendations [8]. The federal government as a whole, working through the White House's Committee on the Environment and Natural Resources, increased funding levels and coordinated research programs to fill the major data gaps [9].

So, a new chapter of toxicological research was opening attempting to define the scope and nature of the endocrine disruptor hypothesis. The U.S. Congress added provisions to the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act of 1996 to require the testing of food-use pesticides and drinking water contaminants, respectively, for oestrogenicity (or estrogenicity) and other hormonal activity. The EPA, with the help of an external advisory committee, **the Endocrine Disruptor Screening and Testing Advisory Committee** (**EDSTAC**), determined that other hormonal activity should include androgens and compounds that affect thyroid function, and expanded the mandate to include all chemicals under EPA's jurisdiction (including the 70,000 chemicals regulated under the Toxic Substances Control Act) [10].

What started out as a hypothesis has become one of the biggest testing programs conceived in the history of toxicology and the only one that has ever been based on mechanism of action as its premise. EPA announced the initial list of chemicals to be screened for their potential effects on the endocrine system (or Tier I testing) in 2009. The list of 73 chemical compounds included known pesticides and high production volume or inert chemicals which can be used as solvents (HPV/inert) Pesticides (selection): Atrazine, Captan, Carbaryl, Carbofuran, Chloropyrifos, Diazinon, Dicofol, Endosulfan, Glyhosate, Malathion, Methyl parathion, Permetin, Resmethtrin, Simazine, BHC (b-hexachlorocyclohexane) etc,

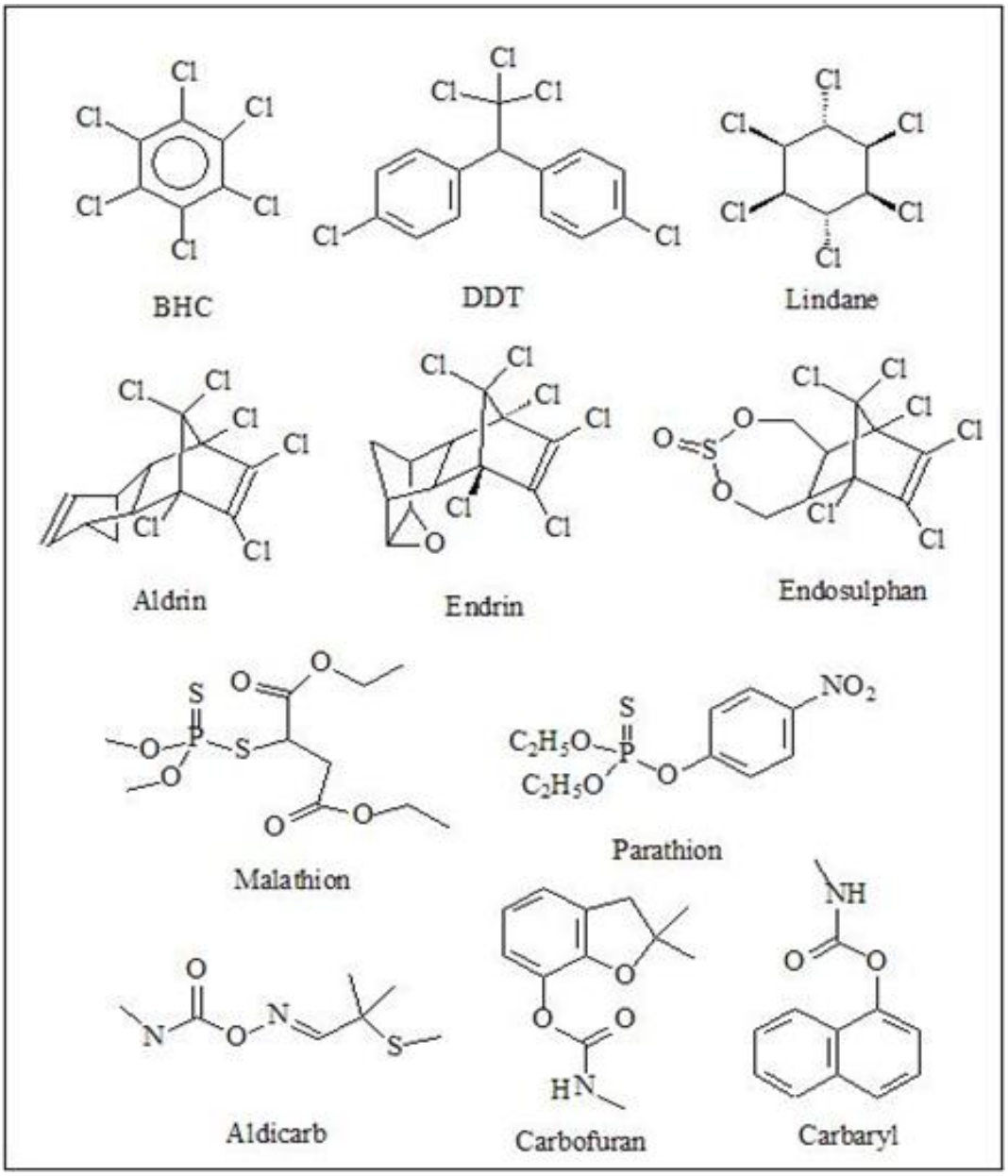


Figure 2. Chemical structures of some pesticides which have endocrine disruption potential. In the last 20 years some of these pesticides have been banned completely or have been restricted for their use on certain crops.

The high production volume chemicals and inerts (mainly solvents) (HPV/inerts) can be used for the preparation of many commercial products: Acetone, Isophorone, Toluene, Butyl benzyl phthalate, Dibutyl phthalate, Diethyl phthalate, Methyl ethyl ketone.

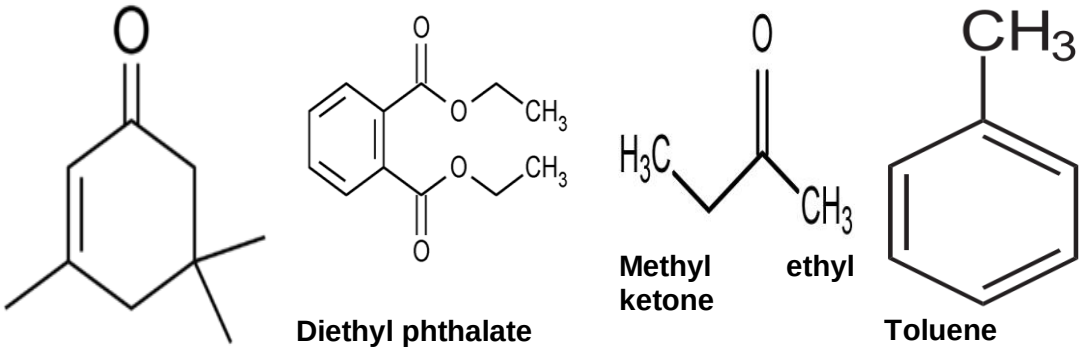


Figure 3. Some organic solvents with endocrine disrupting potential

Testing for EDC potential was eventually expanded to cover all pesticide chemicals, as well as substances that may occur in drinking water. Endocrine disruptor screening is currently proceeding on three fronts: a) selecting chemicals for screening b) testing; and c) implementing the policies and procedures for the Agency. EPA has developed a second list of chemicals for screening.

EPA published the second list of chemicals for Tier 1 Screening in 2010. This list of 134 chemicals includes a large number of pesticides, two perfluorocarbon compounds (PFCs), and three pharmaceuticals (erythromycin, nitroglycerin, and quinoline). This list also consists of an array of other chemicals, ranging from those used for industrial manufacturing processes, as plasticizers, or in the production of pharmaceutical and personal care products (PPCPs). The resulting Tier 2 list contained 134 chemicals: a) Biological agent and naturally-occurring chemicals. b) Chemicals for which the manufacturer, importer or registrant cannot be clearly identified, c) Chemicals already included on the first EDSP list, d) Chemicals that are hormones with confirmed endocrine effects, e) Chemicals that are not likely to be biologically active or which are incompatible with testing assays. f) Pesticides that are scheduled for registration review after 2008. (http://www.epa.gov/endo/pubs/prioritysetting/list2facts.htm).

EPA has summarized the recent studies on EDCs in the following statement. "…In recent years, some scientists have proposed that chemicals might inadvertently be disrupting the endocrine system of humans and wildlife. A variety of chemicals have been found to disrupt the endocrine systems of animals in laboratory studies, and there is strong evidence that chemical exposure has been associated with adverse developmental and reproductive effects on fish and wildlife in particular locations. The relationship of human diseases of the endocrine system and exposure to

environmental contaminants, however, is poorly understood and scientifically controversial (http://www.epa.gov/endo/pubs/edspoverview/whatare.htm).[7, 10, 11].

3. Research Activities on Endocrine Disrupters in Europe and in Japan

The **European Union** (EU) commissioned in 1999 a three-year research programme (COMPREHEND, Community Programme of Research on Environmental Hormones and Endocrine Disrupters). Also, the EU advanced a strategy for short, medium and long-term studies of EDCs. One of the first key short-term actions identified in the European Commission's Communication was the establishment of a priority list of substances for further evaluation of their role in endocrine disruption. The establishment of this list is managed by the Environment DG of the European Commission and is ongoing. Short-term action also encompasses the need for communication to the public (leaflets, press releases, websites) and international co-operation. The European Commission and the **World Health Organisation** (WHO) have co-operated through the **International Programme for Chemical Safety** (United Nations, IPCS) on the maintenance of a global research inventory, housed in Joint Research Center, Ispra-Milano, Italy). The "***Global state-of-the-science of endocrine disruptors"*** report was published in 2002 [12, 13].

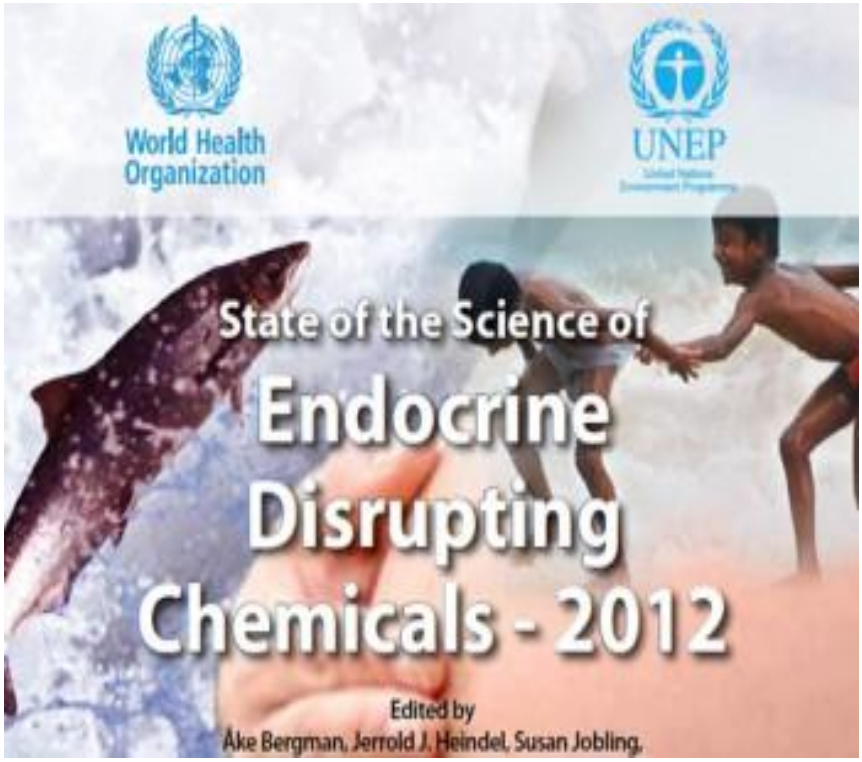
Also, the European Commission supported efforts of the **Organisation for Economic Co-operation and Development** (OECD) to develop agreed test methods for endocrine disrupters [14]. Finally, the EC co-operated with the EPA in the USA to exchange data and research projects on EDCs. The EU funded over 80 research projects, which were part of the 5th, 6th and 7th Framework Programmes for EDC research assessment on human health and wildlife [http://ec.europa.en/research/endocrine/].

The **European Centre for Ecotoxicology and Toxicology of Chemicals** (ECETOC, Brussels) established in 2000 the Environmental Oestrogens Task Force. ECETOX, and proposed a tiered approach for the ecological risk assessment of endocrine disruptors, integrating exposure and hazard (effects) characterization. ECETOX suggested that exposure assessment for EDCs should direct specific tests for wildlife species, placing hazard data into a risk assessment context. Supplementing the suite of mammalian screens now under OECD validation, high priority was given to developing a fish screening assay for detecting endocrine

activity in oviparous species. Taking into account both exposure characterization and alerts from endocrine screening, higher tier tests are also a priority for defining adverse effects. Various proposals were directed to screening of mammals, amphibians, fish reproduction tests, avian risk assessment, aquatic and terrestrial invertebrates [15].

The European Commission organised a conference on "***Endocrine Disruptors: Current Challenges in Science and Policy***" on 11 and 12 June 2012. The presentations and discussions covered the effects of endocrine disruptors on human health and the environment, the risks, the identification of endocrine disruptors and policy objectives The conference provided input to the Commission's upcoming proposal for criteria for the identification of substances with endocrine disrupting properties. (http://ihcp.jrc.ec.europa.eu/ our_activities/food-cons-prod/endocrine_disrupters/eu-conference-on-endocrine-disruptors-2012).

The report on the "**State of the Art of the Assessment of Endocrine Disrupters"** (SOA-AED) has been finalised by the contractor at the end of January 2012 and is available [http://ec.europa.eu/environment/endocrine/ documents/ studies_en.htm]. The fourth implementation report of the Community Strategy for Endocrine Disruptors was published in August 2011 (**SEC(2011) 1001**) (http://ec.europa.eu/environment/endocrine/ documents/sec_2011_1001_en.pdf). A critique of the SOA-AED report in the end of this review.



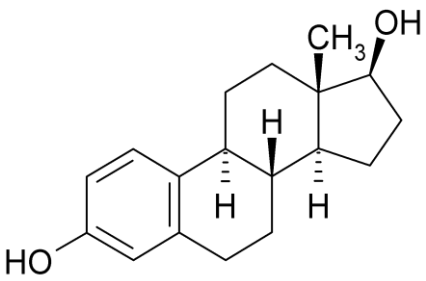
The priority list is now available as an Access-Database. The database comprises not only substances categorised in terms of priority but also the information underlying the prioritisation. (http://ec.europa.eu/environment/endocrine/strategy/short_en.htm).

Japan has been among the first nations to address the issue of EDCs on a national level. In 1997 the Environmental Agency of Japan initiated the programme “Exogenous Endocrine Disrupting Chemicals Task Force”. And in 1998 Japan Ministry of Environment started the SPEED initiative. The aims were: promotion of filed investigations, environmental pollution, adverse effects on wildlife of EDCs, promotion of research and screening, promotion of environmental risk assessment and finally strengthening the international network. The Japan Ministry of Environment worked closely with the WHO/UNEP/ILO International Programme of Chemical Safety [16]

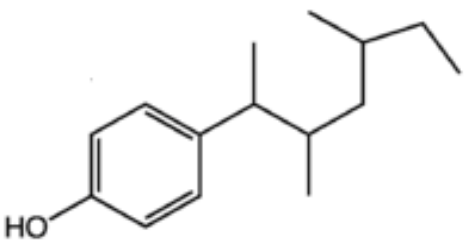
4. Endocrine Disrupting Chemicals (EDCs)

The Endocrine systems (or hormone systems), are found in all mammals, birds, fish, and many other types of living organisms. They are made up of: a. glands located throughout the body, b. hormones that are made by the glands and released into the bloodstream or the fluid surrounding cells and c. receptors in various organs and tissues that recognize and respond to the hormones [17].

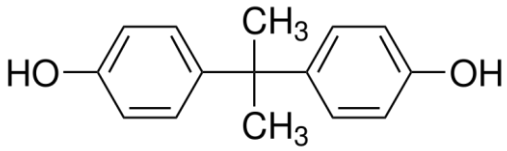
Hormones are released by glands and travel throughout the body, acting as chemical messengers. Hormones interface with cells that contain matching receptors in or on their surfaces. Although hormones reach all parts of the body, only target cells with compatible receptors are equipped to respond. Once a receptor and a hormone bind, the receptor carries out the hormone's instructions by either altering the cell's existing proteins or turning on genes that will build a new protein. Both of these actions create reactions throughout the body. The endocrine system regulates all biological processes in the body from conception through adulthood and into old age, including the development of the brain and nervous system, the growth and function of the reproductive system, as well as the metabolism and blood sugar levels. The female ovaries, male testes, and pituitary, thyroid, and adrenal glands are major constituents of the endocrine system. [18,19].



Estradiol (natural)



Nonyl-phenol



Bisphenol A

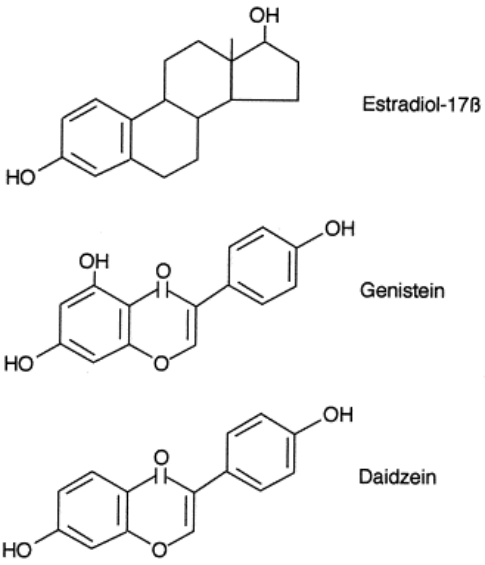
Endocrine disrupting chemicals are substances that can interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for development, behavior, fertility, and maintenance of homeostasis (normal cell metabolism) and one of the nonyl-phenols , an endocrine disruptor and Bisphenol A.

5. Endocrine Disrupting Chemicals in the Environment

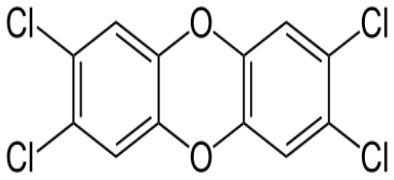
A great number of chemical substances in the past have been suspected of being endocrine disrupters. These include both naturally occurring chemicals (e.g. phytoestrogens) and man-made as pollutants of chemicals used in products (e.g. pesticides, alkylphenols, phthalate esters, etc). Some of the most important EDCs chemicals are.[20-22]

a. **Natural and synthetic hormones** (natural oestrogens or estrogens, synthetic such as oral contraceptives). Meat, dairy products and eggs contain low levels of natural hormones (oestrogens, progesterone, testosterone)

b. **Phytoestrogens** (natural constituents of many foodstuffs, such as beans, sprouts, cabbage, spinach, soybean, etc. Major classes are lignans and isoflavones (e.g. daidzein, genistein).

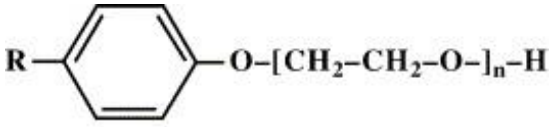


c. **Mycotoxins** (are produced by fungi, e.g. zearalenone is oestrogenic). Food may sometimes be contaminated by oestrogenic mycotoxins.
d. **Organochlorine pesticides** (e.g. DDT, lindane, b-HCH, persistent environmental pollutants). Polychlorinated pesticides have been banned (1970s) or restricted for use for certain crops.
e. **Polychlorinated biphenyls (PCBs)** (chemicals used widely as dielectric and coolant fluids in transformers, capacitors, and electric motors. PCBs' are banned from 1975 because of their environmental toxicity and classification as persistent organic pollutants).
f. **Alkylphenols** (non-ionic surfactants in detergents and paints). Some chemicals: butylphenol, amylphenol, octylphenol, nonylphenol. are used extensively as precursors to the detergents, as additives for fuels and lubricants, polymers, and as components in phenolic resins. These compounds are also used as building block chemicals that are also used in making fragrances, thermoplastic elastomers,
g. **Dioxins.** Polychlorinated dibenzodioxins (PCDDs). Polyhalogenated organic compounds that are significant environmental pollutants. Bioaccumulate in humans and wildlife because of their lipophilic properties, and may cause developmental disturbances and cancer. Dioxins is a by-products in the manufacture of organochlorines, in the incineration of chlorine-containing substances such as PVC (polyvinyl chloride) and from natural sources such as volcanoes and forest fires.

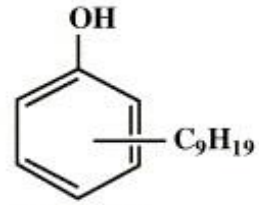


Chemical Structure of *2,3,7,8-tetrachlorodibenzo-p-dioxin* (TCDD)

h. Polyethoxylates (APEs). Herbicides, pesticides and plastics. Breakdown products, such as nonyphenol and octyphenol, are found in sewage and industrial effluents.

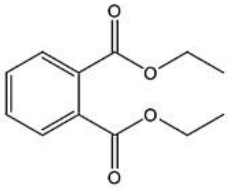


Alkylphenol polyethoxylate

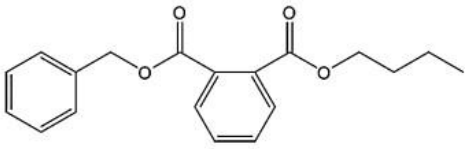


Nonylphenol

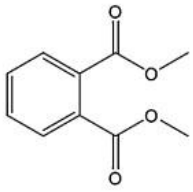
i. Phthalate esters. They are used as plasticizers for PVC. Milk bottles and other plastic items. Common environmental pollutants



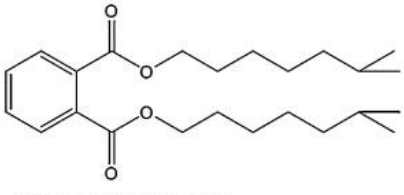
Diethyl phthalate



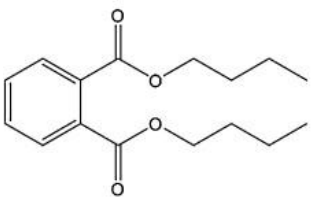
Butyl benzyl phthalate



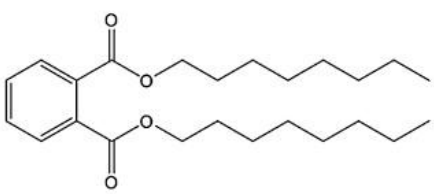
Dimethyl phthalate



Diisooctyl phthalate



Dibutyl phthalate



Dioctyl phthalate

j. **Plastic material** and **food packaging containers** contain chemical substances which can migrate into the food at low level concentrations (e.g. phthalate esters, bisphenol A),

k. **Occupational exposure.** Exposure in the working environment, to lead, manganese and mercury, dibromochloropropane (DBCP), ethylene glycol and carbon disulfide (CS_2) can produce adverse effects on the male reproductive system

l. **Exposure from Drinking water, EDCs in sewage.** Aquatic pollution with EDCs can be a source of exposure for humans and wildlife animals (drinking water, rivers, lakes, sea, sewage, etc).

6. Tests and Methods of Identifying EDCs

In the last twenty years scientists developed various test systems to investigate the ability of chemicals to disrupt the endocrine system [20, 21]. These test methods range from cell-free *in vitro* systems for examining specific hormone receptor binding capacity to multi-generation reproductive studies in experimental animals. The *in vitro* methods have advantages in terms of rapidity, low cost and avoidance of use of experimental animals. But *in vitro* tests have limitations and lack predictions of the outcome of complex interactions in the whole organisms (metabolisms, excretion, interactions). For the definite demonstration of ED disrupting activity it is necessary to perform animal tests with specific validated endpoints of relevance to the health of animal and humans. For comprehensive assessment requires multi-generation studies and examinations of aspects of fertility and reproductive health. These studies are limited. At the moment there is international consensus among scientists on the general strategy for detecting EDCs, although not agreed standardized test methods. [23-25]

The OECD has played a very important part in regulating test strategies and methods for EDCs testing and assessment. The Medical Research Council in England and its Institute for Environment and health (IEH) produced one of the review papers for the OECD on test methods for ECDs [26-28].

The tests of Tier 1 screening referred mainly to *in vitro* assays: estrogen receptor binding/reporter gene assay, androgen receptor binding/reporter gene assay, steroidogenesis assay with minced testis , and *in vivo* tests: Rodent 3-day uterotrohic assay (increase in uterine weight in ovariectomised rat), Rodent 20-day

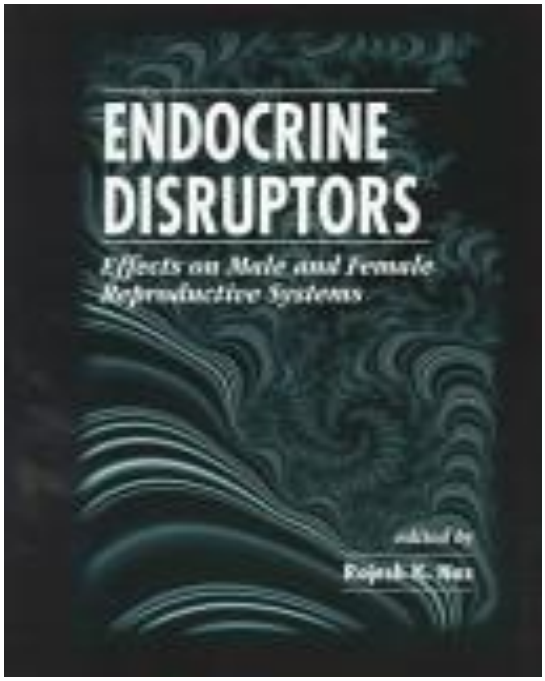
pubertal female with thyroid, Rodent 5-7-day Hershberger assay, Frog metamorphosis assay (rate of tail resorption in *Xenopus laevis,* African clawed frog, a species of South African aquatic frog), Fish gonadal recrudescence assay. These assays are followed with Tier 2 assays to determine and characterize the effects of EDCs chemicals on the endocrine system: two-generation mammalian reproductive toxicity study, Avian reproduction test, Fish-life-cycle test, Mysid (shrimp) life-cycle test and Amphibian development and reproduction test [29].

7. Research Activity and Risk Assessment of EDCs

Risk assessment of EDCs is a difficult problem because of the low level concentrations in the environment. Scientists have to use a range of concentrations wide enough to define the dose-response relationship. The quantitative estimation of the ability of a chemical to cause relevant changes is a difficult aspect for the toxicological assays. Exposure-based risk assessment for the likely impact of environmental EDCs (pollutants) on the health of humans and wildlife must be compared with that of naturally occurring hormones and related substances and then relate this “relative potency” to exposure to the two types of chemicals.

In the last decade there was a high research activity in many developed countries and many publications of report and book on various subjects of Endocrine Disruptors, wildlife species effects, and human health adverse effects, from puberty to various types of cancer. Examples of four books can be found in Figure 4.

Effects of EDCs on human health are especially difficult to estimate because of the food oestrogens. The role of natural estrogens in the diet should also be taken into account when estimate risk. The intake of phytoestrogens from food varies widely among different populations, depending on their dietary habits. The phytoestrogen genistein, for example, with both a higher estrogen activity and higher serum concentrations than other putative EDs, has a relative potency which may even exceed that of estradiol in the case of diets rich in soy. Soy protein (60 g/per day), containing oestrogenic isoflavones, has been shown to prolong the follicular phase of the menstrual cycle in women [30].



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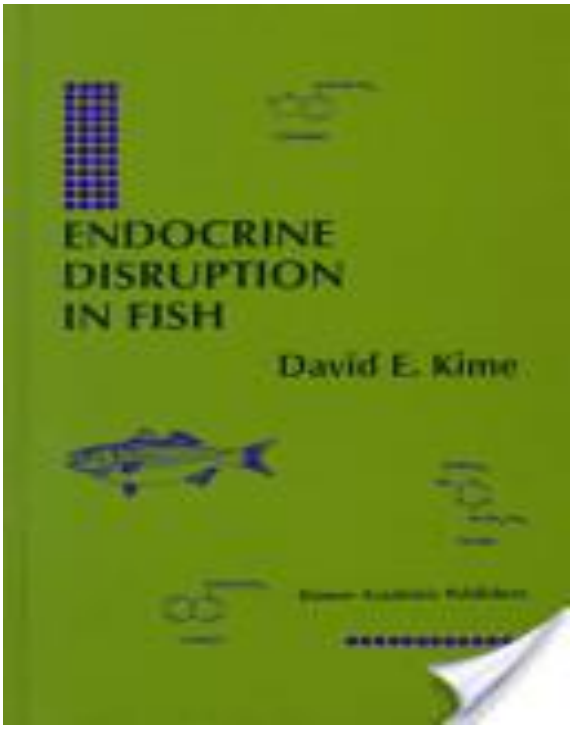

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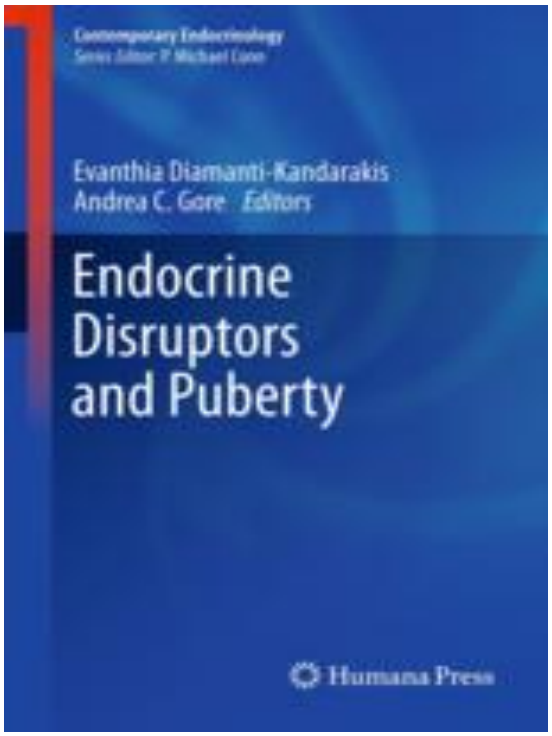

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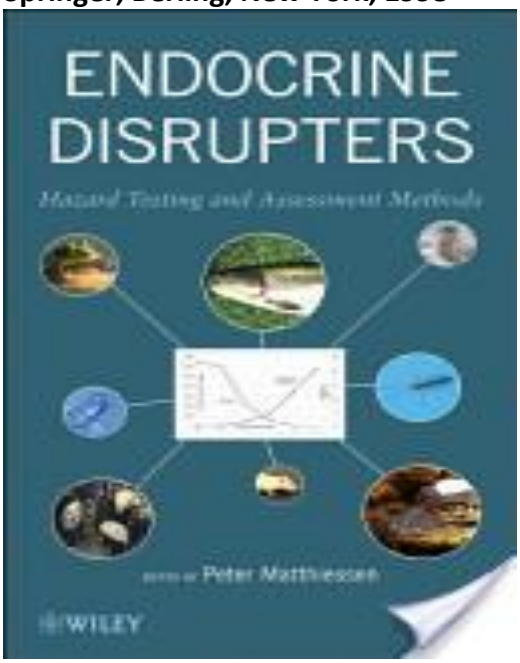

Matthiessen P. Endocrine Disrupters. Hazard Testing and Assessment Methods. John Wiley & Sons, Hoboken, NJ, 2013

Figure 4. In the last decade there were many scientific publications on EDCs

Naturally occurring dietary estrogens have activities ranging from 1/500 to 1/1000 of 17β-estradiol. The estimated daily human intake of estrogenic and anti-estrogenic equivalents, based on in vitro potencies relative to 17β-estradiol (oestrogenicity), has indicated that a woman taking a birth control pill ingests about 16,675 µg equivalents per day, postmenopausal estrogen therapy amounts to 3,350 µg, and ingestion of estrogen flavonoids in food represents 102 µg per day. For comparison, daily ingestion of environmental organochlorine oestrogenic equivalents (like, polychlorinated pesticides) was estimated to be very small , almost negligible (0.0000025 µg) and for TCDD [daily exposure 80-120 pg (pictogram $=10^{-12}$ g) , TEQ-total anti-oestrogen equivalent, 0.00008 µg] [31].

Risk assessment for EDCs is further complicated by the fact that estrogen disrupting substances are mixtures and their potential can be either agonistic or antagonistic in relation to normal hormone activity of an organism. Synergy or additive interactions is another aspect, metabolism (excretion, bioaccumulation, protein binding) and complex endocrine alterations induced by mixtures of chemicals can alter the results. Also, critical periods of sensitivity during the life cycle of an organism may alter the importance of exposure. Many groups of scientists produced results from *in vitro* assays that could not be repeated by others [32-35].

8. Biomarkers and Detection of EDCs

For measuring signal exposure and adverse effects of man-made chemicals with endocrine disrupting properties, scientists can started using various biomarkers which have been developed in the last decades. Biomarkers (or molecular markers) are defined as biological response that can be measured in tissue samples, body fluids or at the level of the whole organism and can be extremely useful means of detecting endocrine disruption *in situ*. Ecotoxicology uses biomarkers as useful indicators of specific environmental pollution by chemicals in an ecosystem. For example, the induction or inhibition of a particular enzyme in an organism, which is linked to growth, reproductive output, viability and resistance to oxidative stress can be useful indicators of levels of environmental pollution. Biomarkers have many positive aspects but also can be misused for measuring environmental contamination [36, 37].

Biomarkers of endocrine disrupting chemicals have been applied to a variety of species. In lower vertebrates (fish and amphibians) production of the egg yolk protein vitellogenin is altered by exposure to xenoestrogens. Vitellin (a vitellogenin

precursor protein) and vitellogenin have been developed as biomarkers of exposure to invertebrates [38, 39]. The identification of phenotypic alteration to an organism in response to endocrine disrupting chemicals may be another important biomarker in field studies. The non-ionic surfactant 4-n-nolnylphenol with strong EDC properties has been shown to cause a range of effects on the life stages of the *Daphnia magna*, such as morphological abnormalities (in prenatal stage) , whilst exposure of adult females led to reduced fecundity [40,41].

Mussels have been used as sentinel organisms for biomonitoring exposure to EDCs in marine environmental, especially estuarine and coastal areas. Mussels (*Mytilus edulis*) were tested for peroxisome proliferation that was assessed by measuring acyl-CoA oxidase (AOX) activity and peroxisomal volume density (Vvp) in digestive glands. [42]. Another example is a study in three species of Mediterranean large pelagic fish and the potential estrogenic effect of polycyclic aromatic hydrocarbons (PAHs) in bluefin tuna, swordfish and Mediterranean spearfish. The biomarkers used were Vitellogenin, zona radiate proteins and mixed function oxidase (EROD, BPMO) as diagnostic tools [43].

9. Mechanisms and Exposure at Different Stages of Organism Development and Dose-response Relationships

Research in the last twenty years has shown that EDCs chemicals can act at multiple sites via multiple mechanisms of action. Receptor-mediated mechanisms have received the most attention, but other mechanisms (e.g., hormone synthesis, transport, and metabolism) have been shown to be equally important. For most associations reported between exposure to EDCs and a variety of biologic outcomes, the mechanism(s) of action are poorly understood.

This makes it difficult to distinguish between direct and indirect effects and primary versus secondary effects of exposure to EDCs. It also indicates that considerable caution is necessary in extrapolating from *in vitro* data to *in vivo* effects, in predicting effects from limited *in vivo* data, and in extrapolating from experimental data to the human situation. A collective weight of evidence is essential in determining under what conditions observed effects resulting from exposure to EDCs occur via endocrine mediated mechanisms. Despite an overall lack of knowledge, there are several examples where the mechanism of action is clearly related to direct perturbations of endocrine function and ultimately to adverse *in vivo* effects [44].

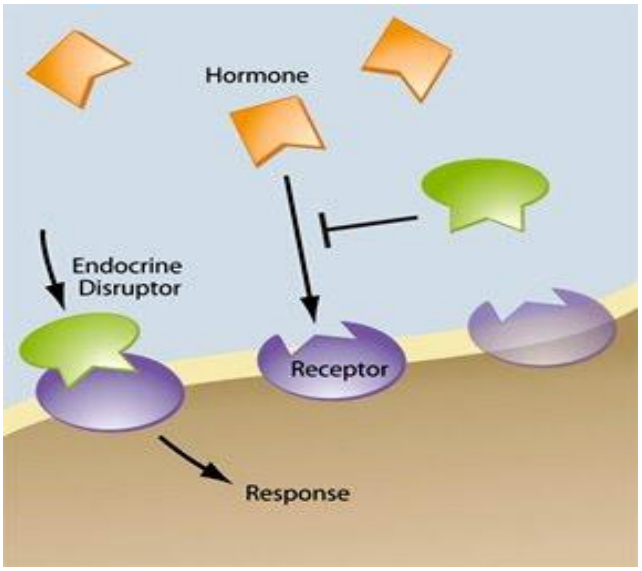

Figure 5. Endocrine disrupting chemicals can bind to a receptor thus preventing the natural hormones to bind and modify their metabolism.

Mechanism of action of endocrine disrupters can be summarized [45]:

a) **Binding and activating the oestrogen receptor** (therefore acting as an oestrogen

b) **Binding but not activating the oestrogen receptor** (therefore acting as an anti-oestrogen)

c) **Binding other receptors**

d) **Modifying the metabolism of natural hormones** (Some chemicals, such as lindane and atrazine, can effect the metabolic pathway of oestradiol, producing more oestrogenic metabolites. Other chemicals activate enzymes which speed up the metabolism of hormones, so disrupting their natural state.

e) **Modifying the number of hormone receptors in a cell**

6) **Modifying the production of natural hormones**

The time of exposure of endocrine disrupting chemicals in the various stages of development of an organism is very vital for the adverse effects. Exposure to EDCs during the period of “programming” of the endocrine system may result in a permanent change of function or sensitivity to stimulatory/inhibitory signals. Exposure in adulthood may be compensated for by normal homeostatic mechanisms and may therefore not result in any significant or detectable effects. Exposure to the same level of an endocrine signal during different life history stages or during different seasons may produce different effects. Because of cross talk between different

components of the endocrine systems, effects may occur unpredictably in endocrine target tissues other than the system predicted to be affected. Scientists have gathered considerable data on the early molecular events involved in hormone response to various organisms, but there is little knowledge of the relationship between these molecular events and the potential for adverse health outcomes. Mechanisms for EDCs effects are very important. Until such data become available, it will remain difficult and controversial to attribute adverse effects due to endocrine-mediated pathways under various pollution regimes in the aquatic or terrestrial environment. Due to the emphasis on mechanisms of action (MOA), consideration of EDCs in current testing and regulatory frameworks has been challenging [46].

Another very difficult scientific issue with EDCs effects is the dose-response relationships and perhaps it is the most controversial issue among scientists. Timing of exposure is also absolutely critical to the understanding of dose–response relationships for EDCs for both wildlife and humans and for cancer as well as for developmental, reproductive, immunological, and neurological effects. The National Toxicology Program (USA) on the request of the EPA studied the issue of low-dose of EDCs. The peer-review panel considered mechanistic data that might influence the plausibility of low-dose effects for EDCs and identified study design issues or other biologic factors that might account for differences in reported outcomes among studies. The panel found that low-dose effects have been demonstrated in laboratory animals exposed to certain endocrine-active agents. In some cases where low-dose effects have been reported, the findings have not been replicated. The shape of the dose-response curves for reported effects varied with the end point and dosing regimen and were low-dose linear and threshold-appearing. The findings of the panel indicate that the current testing paradigm used for assessments of reproductive and developmental toxicity should be revisited to see whether changes are needed regarding dose selection, animal-model selection, age when animals are evaluated, and the end points being measured following exposure to endocrine-active agents [47].

10. EDCs and Adverse Effects in Wildlife Species

Toxicological and ecotoxicological studies have shown that exposure to certain EDCs has contributed to adverse effects in some wildlife species and populations. Similar studies have been performed under laboratory conditions at increased concentrations of EDCs to evaluate the adverse health effects on

experimental animals. The endocrine disrupting effects vary from subtle changes in the physiology and sexual behaviour of species to permanently altered sexual differentiation. Most of the data come from Europe and USA. Aquatic species (at the top of the food chain) are most affected, but effects have also been observed in terrestrial species (birds, mammals). Some adverse effects observed in certain species are likely to be endocrine mediated, but in most cases, the causal link between exposure and endocrine disruption is unclear [48-51].

Exposure to EDCs and Mammals*:* Exposure to organochlorines (PCBs, DDE) has been shown to adversely impact the reproductive and immune function in Baltic seals, resulting in marked population declines. These seals exhibit a compromised endocrine system, but precise mechanisms of action remain unclear [52, 53]. The effects of global change on biodiversity and ecosystem functioning encompass multiple complex dynamic processes. Climate change and exposure to EDCs are currently regarded as two of the most serious anthropogenic threats to biodiversity and ecosystems. Scientists, therefore, are especially concerned about the possible effects of EDCs on the ability of Arctic marine mammals and seabirds to adapt to environmental alterations caused by climate change [54].

Birds*:* Bioconcentration of polychlorinated pesticides (especially DDT which is persistent and lipoplilic) in birds of prey, occurs because they are high on food chains, but also because predatory birds tend to live a long time. DDT and its metabolite DDE alter the bird's calcium metabolism in a way that results in thin eggshells. Heavily DDT-infested birds of prey decreased dramatically since the eggshells were unable to support the weight of the incubating bird [55, 56]. Scientists were alerted to the fact of ecotoxicological damage through the bioaccumulation of persistent polychlorinated pesticides and PCBs. Eggshell thinning and altered gonadal development have been observed in birds of prey and fish-eating birds. A syndrome of embryonic abnormalities (known as GLEMEDS) has been observed in fish-eating birds (Great Lakes, USA) and can be directly related to PCB exposure, but the precise linkage to endocrine function is uncertain [57,58].

Reptiles: Reptiles have been used as bioindicators of environmental pollutants affecting wildlife. Scientists recognized that reptiles have the potential to act as good biomodels to elucidate the mechanisms of endocrine disrupters due to the fact that different species of reptiles have varying modes of gender determination (genotypic sex determination or temperature-dependent sex determination) and parity modes (oviparity or viviparity). Laboratory studies of oviparous reptiles with temperature-dependent sex determination reveal that embryonic exposure to natural hormones

and many man-made chemicals (including the ubiquitous PCBs and organochlorine pesticides) [59].

A presumed pesticide spill in Lake Apopka (Florida, USA) provided the opportunity for scientists to study a well-publicized example of potential EDC effects on population decline in alligators. A variety of gonadal and developmental abnormalities were observed that have been attributed to high levels of various organochlorine contaminants that disrupt endocrine homeostasis. Several hypotheses have been proposed to explain the contaminant-induced endocrine disruption, but the precise cause(s) is not known [60].

Figure 6. Lake Apopka (Florida, USA) and American alligator (*Alligator mississippiensis*)

A study examined the reproductive and developmental endocrinology of several populations of American alligator (*Alligator mississippiensis*) living in contaminated and reference lakes and used as a sentinel species in field studies for endocrine disrupters. It observed that neonatal and juvenile alligators living in pesticide-contaminated lakes have altered plasma hormone concentrations, reproductive tract anatomy and hepatic functioning. Also, experimental studies exposing developing embryos to various persistent and non-persistent pesticides, have produced alterations in gonadal steroidogenesis, secondary sex characteristics and gonadal anatomy. An understanding of the developmental consequences of endocrine disruption in wildlife can lead to new bioindicators of exposure and a better

understanding of the most sensitive life stages and the consequences of exposure during these periods [61].

The same study with alligators in Lake Apopka was extended to investigate whether bone tissue, known to be affected by sex steroid hormones, is a potential target of endocrine disruptors. Long bones from 16 juvenile female alligators from Lake Apopka and from Lake Woodruff (control lake) were evaluated by peripheral quantitative computed tomography. Scientists observed significant differences in bone composition, with female alligators from the contaminated lake having greater trabecular bone mineral density (BMD), total BMD and trabecular mineral content compared to control alligators. The results suggest that juvenile female alligators from Lake Apopka were exposed to contaminants that created an internal environment more estrogenic than that normally observed [62].

Amphibians*:* It is evident that scientists will direct their research on organisms in the surface water which are the most important environmental matrix to be polluted by EDCs. Thus, aquatic vertebrates such as fishes and amphibians are the most endangered. Population declines in amphibians has been observed in both pristine and polluted habitats worldwide. After numerous studies scientists concluded that currently the data are insufficient to implicate EDCs as causative agents.

Declines in amphibian populations (frogs, toads, salamanders, newts , and caecilians) have been noted since the 1980s at various locations all over the world. These declines were perceived as one of the most critical threats to global biodiversity, and several causes are believed to be involved. Studies showed that the most serious causes were diseases (mainly infectious), habitat destruction and modification, exploitation, pollution form pesticides and EDC chemicals, introduced species, and increased ultraviolet-B radiation (UV-B). However, many of the causes of amphibian declines are still poorly understood, and the topic is currently a subject of much ongoing research [63, 64]. The 2004 Global Amphibian Assessment found that 32% of species were globally threatened, at least 43% were experiencing some form of population decrease. In 2010, the **IUCN Red List** (and updates) lists 486 amphibian species as "Critically Endangered". [65].

There is considerable evidence from scientific observations that parasitic (*trematode platyhelminths*, a type of fluke) have contributed to developmental abnormalities and population declines of amphibians in some regions without apparent pollution of EDCs [66,67]. A recent review described the findings of research in the UK and Japan concerning the association of EDCs and amphibians. Laboratory studies of the effects of single chemicals on endocrine-relevant endpoints

in amphibian, mainly anuran (order of frogs), models are valuable in characterizing sensitivity at the individual level and may yield useful bioassays for screening chemicals for endocrine toxicity. Nevertheless, in the UK and Japan as in many other countries, it has yet to be demonstrated unequivocally that the exposure of native amphibians to EDC environmental contaminants results in adverse effects at the population level. Scientists suggest that assessing the potential of such effects is likely to require an eco-epidemiological approach to investigate associations between predicted or actual exposure of amphibians to EDCs and biologically meaningful responses at the population level [68].

Figure 7. Global amphibian population decline (especially frogs), and deformities or malformations gave the impression that environmental pollution was vastly responsible than other causes.[*Blaustein, AR, Johnson, PTJ. The complexity of deformed amphibians. Frontiers in Ecology and the Environment 1: 87-94, 2003. Blaustein AR, Johnson, PTJ. Explaining frog deformities. Scientific American 288: 60-65, 2003].*

Despite numerous reports on EDCs in fishes, information about EDCs in amphibians is scarce, and this paucity of information is of particular concern in view of the worldwide decline of amphibians. EDCs could contribute to changes of amphibian populations via adverse effects on reproduction and the thyroid system. In amphibians, EDCs can affect reproduction by (anti)estrogenic and (anti)androgenic modes of action that produce severe effects including abnormal sexual differentiation. EDCs actions on the thyroid system cause acceleration or retardation of metamorphosis, which may also affect populations. In

particular, effects of EDCs on the thyroid system triggering metamorphosis can be determined easily and most sensitively in amphibians compared to other vertebrates. Effects of EDCs on the thyroid system of amphibians can be assessed by a single animal model (*Xenopus laevis*), whereas the various types of reproduction need comparative studies to investigate whether general endocrine principles do exist among several species of anurans and urodeles [69].

Fish*:* There is extensive evidence that chemical constituents present in pulp and paper mill effluents and sewage treatment effluents can affect reproductive endocrine function and contribute to alteration in reproductive development in fishes. A variety of mechanisms (e.g., hormone–receptor interactions, interference with sex steroid biosynthesis, altered pituitary function) are involved, but precise modes of action or the causative chemicals are still poorly understood.

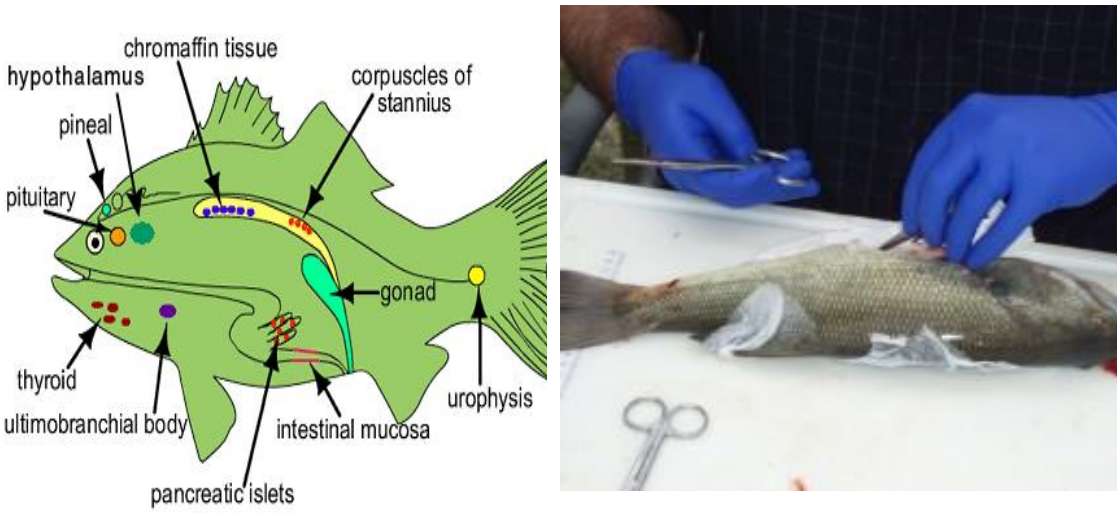

Figure 8. EDCs affect fish by arious endocrine mechanisms

The potential of EDCs to impact the reproductive health of various fish species is open to experimental challenges. Overall, data from laboratory experiments support the hypothesis that EDCs in the aquatic environment can impact the reproductive health of various fish species, but evidence that EDCs in the aquatic environment are actually impacting the reproductive health and sustainability of indigenous fish populations is less convincing. The scarcity of evidence linking impacts of environmental EDCs with changes in reproductive success of indigenous fish populations may reflect a critical need for a dependable method or

indicator to assess reproduction of fish in situ. Linking EDCs and reproductive impairment with an ecologically relevant impact on the sustainability of real fish populations remains, with few exceptions, an open challenge [70].

A review in 2011 focused on the growing evidence that EDCs interfere with the fish immune system. Research in the last years showed that fish that have been exposed to EDCs are more sensitive to pathogens during gametogenesis. Also, it has been shown that sex-steroid-like endocrine disruptors in fish have advanced specificity on the fish immune system in comparison to mammals. The recent literature suggests that immune parameters may be used as biomarkers of contamination by EDCs. However, caution should be used in the assessment of such immunotoxicity. In particular, more attention should be paid to the specificity of these biomarkers, the external/internal factors influencing the response, and the transduction pathways induced by these molecules in fish. The use of the well-known mammalian models provides a useful guide for future research in fish [71].

A review by Scholz and Kluver (2009) summarised and categorised the experimental evidence that links disruption of gonadal development in gonochoristic fish with reference to contaminations by endocrine disrupting chemicals. The review referred to laboratory studies using water-borne exposures and histopathological analysis. Parameters ranging from simple quantitative characteristics such as sex ratio, number of sex reversed fish, and gonadosomatic index (GSI) to detailed morphometric analyses have been considered. [72].

Evidence for altered physiology in fish as a consequence of endocrine disruption were presented in a recent review. Research showed that some of the most widely reported effects in fish were directed on sexual development and function. Fish behaviors can also be affected by EDCs which potentially has wide implications for individual fitness and population level outcomes. The review presented a critical assessment on reported effects of EDCs on behavior in fish, focusing on behaviors associated with reproduction. Adopting behaviors in fish as indicators of chemical exposure and effects, however, still has many technical and interpretation challenges and there is little information available on how behaviors under laboratory conditions equate with those occurring in wild populations [73].

Invertebrates: Exposure of marine gastropods to TBT (a biocide used in antifouling paints, TriButyl Tin) provides the clearest example in invertebrates of an endocrine-mediated adverse effect caused by exposure to an environmental contaminant. Masculinization of marine gastropods exposed to TBT has resulted in worldwide

declines of gastropods. The endocrine mechanism probably involves elevated androgen levels possibly through altered aromatase activity [74].

Examples of endocrine disruption in marine invertebrates, including imposex in gastropod mollusks can be found in the scientific literature. It appears that is the result of exposure to organotin compounds and intersex in crustaceans exposed to sewage discharges. Laboratory data concerning the effects of endocrine disruptors on the growth and reproductive output of the deposit feeding amphipod *Corophium volutator* and the polychaete worm *Dinophilus gyrociliatus*. The ecological significance of EDCs in marine invertebrates has been discussed in reports [75].

Imposex is one of the best documented examples of endocrine disruption in wildlife. It is characterized by the superimposition of male characteristics, such as a penis and a vas deferens, onto females of marine gastropods. Ever since it was first described in *Nucella lapillus* (1970) numerous studies have been published on the subject. A clear association between exposure to tributyltin (TBT), the active ingredient in antifouling paints, and imposex has been demonstrated for several species. At least 195 species of prosobranch gastropods are known to be affected, albeit the mechanisms responsible are not known. A basic understanding on mollusk endocrinology is still today far from achieved, which hinders scientists from comprehension of the imposex process [76].

Studies in wildlife have been proposed as “sentinels” of human exposure to EDCs. However, given the diversity of wildlife, caution must be taken in extrapolating the responses to EDCs, as research has focused primarily on only a few species of wildlife. Also, potential effects of EDCs on wildlife tend to focus on the individual, whereas ecological risk assessments focus on populations and communities. Overall, the current scientific knowledge provides evidence that certain effects observed in wildlife can be attributed to chemicals that function as EDCs. However, in most cases, the evidence of a causal link is weak, and most effects have been observed in areas where chemical contamination is high.

11. Conclusions Wildlife Exposures to EDCs

In this section we presented selected studies in the last 20 years. All these studies that provided data for scientists show clearly evidence that considerable exposure (for long time and at high concentrations) to potential EDCs (particularly Persistent Organic Pollutants, POPs) has occurred in a variety of wildlife species. However, most of these exposure data come from selected species at the top of the

food chain or from wildlife living in a highly polluted habitats in Europe and in North America. Exposure data for non-persistent EDCs, for other wildlife species, at low environmental levels, and in other parts of the world are generally lacking. Although these results have important scientific value, there are difficulties in comparing exposure levels between species, over time, and in different areas because of different approaches to sampling, analytical methodologies, data reporting, and statistical treatment [77, 78].

Scientists suggest that in order to have adequately assess EDC related effects in wildlife, we must aim for global, long-term exposure monitoring, using harmonized and consistent methodologies to ensure comparability of data. This is a serious problem until now and some conclusions are premature in blaming EDC chemicals with adverse effects in wildlife.

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Typical Examples of Persistent EDCs in Wildlife (Baltic Sea, Great Lakes in USA, and the Arctic region (data from multiple research projects)

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Baltic Sea :

Environment: Marine enclosure, little clean water dilution, strong halocline; temperate climate,

Chemicals : DDT, PCBs, HCB, PCDDs, PCDFs, PBDEs, HCHs DDT, PCBs**,**

Sources of pollution: Highly industrialized communities within Some areas of shoreline highly industrialized areas on periphery; drainage areas

Examples of species affected : Fish: Salmon, **Birds** : White-tailed sea eagle, guillemot, razorbill **, Mammals** : Gray seal, otter, mink

Great Lakes (USA): Connected freshwater lakes;

Environment: temperate climate lakes

Chemicals : PCDDs, PCDFs, PBDEs, DDT, PCDDs,

Sources : drainage areas industrialized long-range transport for some pollutants

Examples of species affected: Fish : Lake trout **,** chinook salmon **, Birds:** Herring gulls, Forster's terns, double-crested cormorants white-tailed eagle

Mammals : Mink

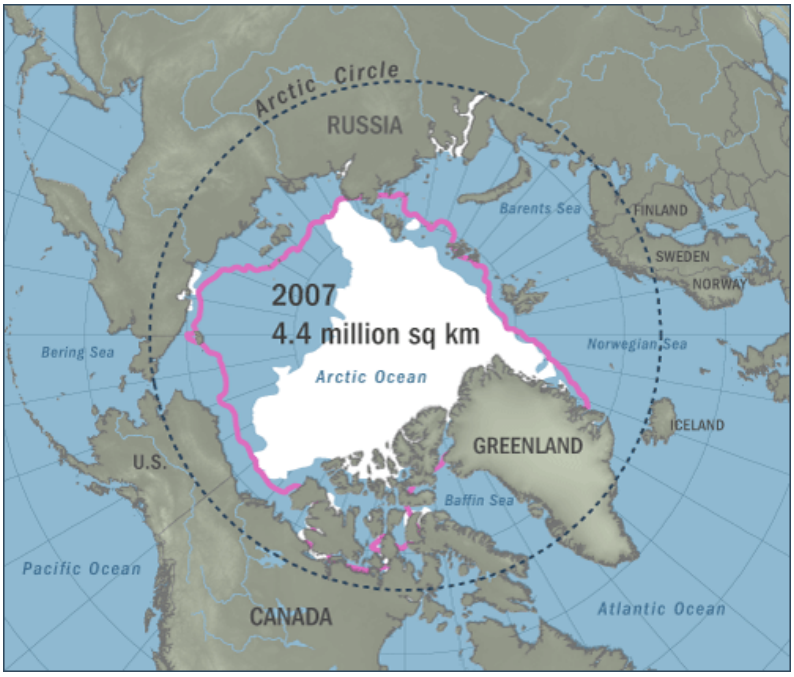
Arctic Region:

Environment : Dramatic seasonal differences; polar seas and covered by ice for much of the year

Chemicals : DDT, PCBs (including OH-PCBs), PCDDs, PCDFs

Sources : Highly industrialized areas ; long-range transport for some pollutants

Examples of species affected : , Fish: Arctic char, **Birds** : Glaucous gull, thick-billed murre, puffin, white-tailed eagle**, Mammals** : Mink,



12. Environmental Pollution by EDCs and Human Health Effects

Environment awareness of toxic chemicals in the 1970s and 1980s was spreading among the population of the industrialised societies. It was inevitable to cause alert when the hypothesis of environmental EDCs and their risk concerning. The scientific findings of EDCs in the aquatic environment and the wastewater treatment plants inevitably raised the level of concern among environmentalists and health practitioners for possible adverse health effects on humans and especially children.

But, the majority of studies on humans for adverse health effects of EDCs at environmental concentrations (air, aquatic environment, drinking water, food, etc) has so far failed to provide firm evidence of direct causal associations between low-level (i.e., levels measured in the general population) exposure to chemicals with EDC potential and adverse health outcomes. This is a general conclusion of experts in the international report (international programme on chemicals WHO, ILO, UNEP, 2002) [50]. (http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/)

Scientists concluded that it is difficult to compare and integrate results from diverse human studies, because data are often collected at different time periods, using different experimental designs and under different exposure conditions. Often exposure data are completely lacking. Of particular concern is the lack of exposure

data during critical periods of development that influence later functioning in adult life. Furthermore, the concentrations and potencies of endogenous hormones and phytoestrogens (food constituents) are generally higher than those of exogenous chemicals. Despite these difficulties, exposure to EDCs has been suggested to play a role in adverse health outcomes, and concerns remain [50].

WHO publication, Geneva, 2012

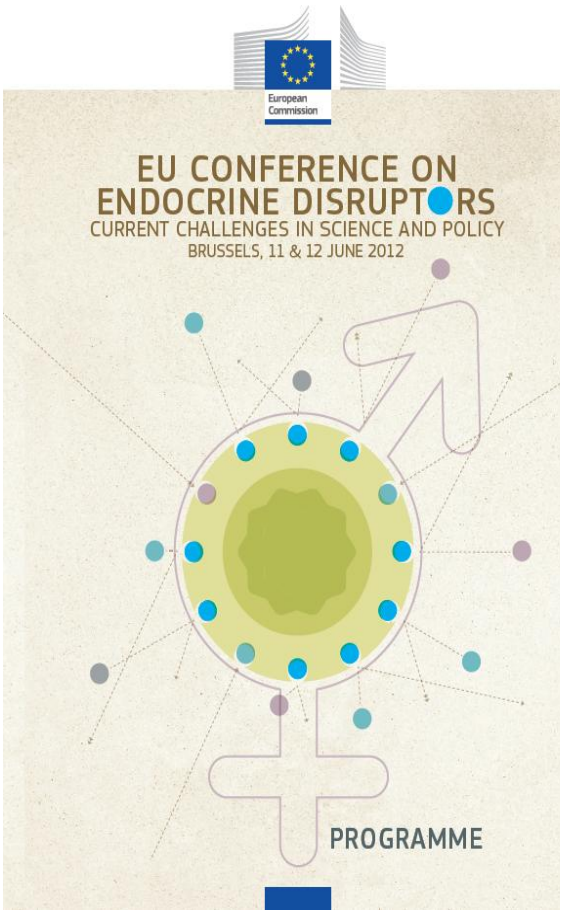


Figure 9. WHO: "**Possible Developmental Early Effects of EDs on Child Health**" (2012), and **European Union Conference on Endocrine Disruptors, Brussels 11 & 12 June, 2012.**

Analysis of the human data by itself, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low-level (i.e., environmental levels measured in the general population) exposure to chemicals with EDCs and adverse health outcomes. In the EDC case scientists are interested with risk assessment of chemical mixtures, combined and cumulative exposures. We understand that there is a lack of knowledge on how to monitor effects of complex

exposures, and there are few reviews on biomonitoring complex exposures. Scientists put emphasis on endocrine disrupters acting via epigenetic mechanisms and on carcinogens. Solid evidence shows that these groups of chemicals can interact and even produce synergistic effects. They may act during sensitive time windows and biomonitoring their effects in epidemiological studies is a challenging task [79].

Reproductive Effects (sperm quality) and EDCs *:*

Many years ago a number of studies reported a decline (since the 1930s) in human sperm quality in several countries. There are important variations in sperm count, but there are no firm data that directly addressed the possible cause and effect relationship between declining sperm quality and exposure to EDCs. Several meta-analyses of existing studies reached different conclusions, and the issue remains controversial. Even if there has been deterioration in semen quality, this would not necessarily be due to endocrine disruption [80, 81]. The evidence for association between levels of exposure found in the general population and serious adverse effects on male reproduction, including fertility, is still lacking. A recent European Union-supported study, on the effect of persistent organohalogen pollutants on human reproduction, failed to show any correlation between post-natal exposure levels and fertility [82].

EDCs can mimic natural hormones, inhibit the action of hormones, or alter the normal regulatory function of the endocrine system and have potential hazardous effects on male reproductive axis causing infertility. Although testicular and prostate cancers, abnormal sexual development, undescended testis, chronic inflammation, Sertoli-cell-only pattern, hypospadias, altered pituitary and thyroid gland functions are also observed, the available data are insufficient to deduce worldwide conclusions. Many well-controlled clinical studies and basic scientific discoveries in the physiology, biochemistry, and molecular and cellular biology of the male reproductive system have helped in the identification of greater numbers of men with male factor problems [83]. Available human and experimental animal studies demonstrate that high-level exposure to certain environmental chemicals can impair fertility and increase the rate of spontaneous abortion, but the relationship to endocrine disruption remains speculative. Declining sex ratios (fewer males) have been recorded in a number of regions and countries, and there is evidence that unidentified external influences are associated with such changes, but the mechanism(s) is unknown. Temporal increases in the frequency of development

abnormalities of the male reproductive tract, particularly cryptorchidism and hypospadias, have been reported, but the role of exposure to EDCs is unclear [84, 85].

EDC Exposure and Endometriosis*:*

Exposure to certain EDCs has been reported to be associated with endometriosis, but the studies remain equivocal. Endometriosis is an estrogen-dependent gynecological disorder associated with pelvic pain and infertility. (occurs in 6–10% of women and up to 50% of women with pelvic pain and infertility). There are suggestive animal data of adult exposure to EDCs and development of or exacerbation of existing disease, and there is evidence that *in utero* exposure in humans to DES results in an increased relative risk = 1.9 (95% confidence interval, 1.2–2.8). Most striking are the observations of rhesus monkeys administered different doses of TCDD and their subsequent development of endometriosis [86-89]. A recent review collected the most significant papers regarding the interaction among endometriosis, hormones and genetic polymorphic variants. Scientists agree that many aspects of female reproductive function are strongly influenced by genetic factors and numerous studies have attempted to identify susceptibility genes for disorders affecting female fertility such as endometriosis. The importance of steroid hormones on endometriosis is unquestionable. The disease is most prevalent in women of reproductive age and regresses after menopause. Sex steroids, estrogen and progesterone, are mainly produced in the ovaries and they regulate the growth of endometrial tissue. In addition, estrogen plays an important role in the regulation of cyclic gonadotropin release and in folliculogenesis. Numerous studies have been conducted to demonstrate the interaction of hormone and their receptors with endometriosis with conflicting results. Besides, environmental chemicals, known as endocrine disruptors, which have the capacity to mimic, block or modulate the endocrine system through the interaction with steroidal receptors. Recently evidences have proposed a putative role for ubiquitous environmental contaminants in the occurrence of endometriosis [90].

EDC Exposure and Precocious (Timing) Puberty*:*

In the last decade concerns have been raised about the influence of EDCs on the timing of puberty, but the possible mechanisms of action and role of other factors such as nutrition need to be clarified. A recent review presented data on adverse health and reproductive outcomes that have been attributed to estrogen

disruptors in laboratory animals with regard to puberty timing. Puberty is regulated by the endocrine system. Disruption of that system by exposure to environmental hormone-mimicking substances such as endocrine disruptors, may affect this development profoundly. In the last decades there has been a great secular trend in the earlier timing of puberty such as both puberty onset and menarche age.

Scientists suggested that these changes caused by environmental factors such as improved socioeconomic status, better healthcare and improved nutrition. However, part of the phenomenon could be associated with exposure to endocrine disruptors because of their estrogen activity or increase endogenous sex hormone levels. These EDC pollutants tend to degrade slowly in the environment, to bioaccumulate in the food chain and to have long half-lives in humans. Studies showed that endocrine disruptor environmental chemicals (xenoestrogens) can disregulate hypothalamic-pituitary-gonadal axis potentially inducing reproductive disorders. In the past there were several case reports of accidental exposure to estrogenic compounds in cosmetic products, food and pharmaceuticals. The outbreak of epidemics of premature thelarche in some geographical areas has also been suggested to be linked to exposure to oestrogen disrupters such as dioxins, furans and organohalogens [91].

The high incidence of precocious puberty in foreign children migrating to Belgium and the detection in their plasma of a long-lasting 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT) residue suggest the potential role of environmental endocrine disrupting chemicals in the early onset of puberty. This hypothesis was confirmed by experimental data showing that temporary exposure of immature female rats to DDT in vivo results in early onset of puberty. Pathological variations in the timing of puberty may provide unique information about the interactions of either environmental conditions or genetic susceptibility with the hypothalamic mechanism controlling the onset of sexual maturation, as shown by examples of precocious puberty following exposure to endocrine disrupters or due to hypothalamic hamartoma [92].

Neurological Development and EDCs*:*

Data from human and experimental animal studies clearly indicate that exposure (particularly prenatal exposure) to certain EDCs (e.g., PCBs) can have adverse effects on neurological development, neuroendocrine function, and behaviour. Some of these effects appear to result from altered thyroid or neurotransmitter function, but in most instances endocrine mechanisms have not

been demonstrated. Similar effects can also result from exposure to chemicals that induce developmental neurotoxicity but have no known endocrine action [93-95].

EDCs Exposure and Immune Function*:*

Exposure to environmental chemicals, including certain EDCs, has been shown to alter immune function in humans and animals. However, it is not clear whether such impaired function is due to endocrine-mediated mechanisms. Scientists found that chemical exposures during development can alter disease susceptibility later in life. Endocrine disrupting chemicals (EDCs) can produce adverse developmental, reproductive, neurological, cardiovascular, metabolic and immune effects in humans. In addition, EDCs interfere with the synthesis, secretion, transport, activity, or elimination of natural hormones [96-98].

13. Is EDC Exposure Connected with Increased Risk for Cancer?

Temporal increases in the incidence of certain cancers in hormonally sensitive tissues in many parts of the industrialized world are often cited as evidence that widespread exposure of the general population to EDCs (especially pesticides and polychlorinated chemicals). These increases cannot be adequately explained by improved diagnostic techniques, and it has been argued that these trends coincide roughly with the increased use and release of industrial chemicals into the environment [50].

It is known that oestrogen and xenoestrogens mediate critical points in carcinogenesis by binding to oestrogen receptors, whose distribution is age-, gender-, and tissue-specific. A recent review collected data about cancer types whose causes may be found in environmental exposure to xenoestrogens. Cancer types that have been well documented in literature to be related with high or persistent environmental exposure include the reproductive system, breast, lung, kidney, pancreas, and brain. The results showed a significant correlation between exposure to xenoestrogens and increased, gender-related, cancer risk and a need to re-evaluate agents so far defined as endocrine disruptors, as they are also key molecules in carcinogenesis [99, 100].

Epidemiological Studies of Breast Cancer and EDC*:*

In the last 20 years numerous human epidemiological studies and experimental laboratory studies have been conducted to determine whether

environmental EDCs may contribute to an increased risk of breast cancer. Despite the initial positive results of a connection with increased risk, more research and better data showed that current scientific evidence does not support a direct association between exposure to environmental EDCs and increased risk of breast cancer [50]. However, there are studies that measured EDC exposure levels in adult women. But data on exposures during critical periods of development are lacking. Adult women currently at risk for breast cancer may have been exposed to exogenous EDCs *in utero* or during infancy, childhood, and adolescence in the mid twentieth century when contaminant levels of organochlorines were higher. Although the incidence of breast cancer has increased over the past 50 years, there are multiple causes and hormonal interactions involved. Risk factors for breast cancer may be classified into four broad categories: (1) genetic/familial, (2) reproductive/hormonal, (3) lifestyle, and (4) environmental (including EDCs). Established risk factors for breast cancer include older age, later age at first full-term pregnancy, no full-term pregnancies, postmenopausal obesity, and genetic factors. However, these known risk factors cannot account for the majority of cases [101].

In the early 1990s, it was suggested that exposure to some environmental chemicals such as organochlorine compounds may play a causal role in the etiology of breast cancer through estrogen-related pathways. The relationship between organochlorines and breast cancer risk has been studied extensively in the past decade and more, and at this point there is no clear evidence to support a causal role of most organochlorine pesticides in the etiology of human breast cancer, but more evidence is needed to assess risk associated with polychlorinated biphenyls (PCBs). Future studies need to consider the combined effects of exposures, concentrate on vulnerable groups [101, 102].

Most of the epidemiological studies in Western countries focused on the association between breast cancer risk and exposure to organochlorine pesticides or polychlorinated biphenyls (PCBs), which are EDCs and potential risk factors for human breast cancer, but they were negative. A large study was contacted in Japan. Serum samples were measured for PCBs and nine pesticide-related organochlorines, including (DDT). No increase in the risk of breast cancer was seen among women with higher serum concentrations of any organochlorine pesticides (o,p'-DDT, p,p'-DDT, p,p'-DPD, hexachlorobenzene, b-hexachlorocyclohexane, trans-nonachlor, cis-nonachlor, oxychlordane, mirex, or PCBs. Rather, higher serum levels of cis-nonachlor, mirex, or total PCBs were associated with a decreased risk of breast cancer. Overall, these results suggest that breast cancer risk in Japan, a low-

incidence country, is similar to that in western countries in terms of organochlorine exposure [103].

EDC Exposure and Endometrial, Testicular, Prostate and Thyroid Cancer*:*

Limited available data do not support a causative role for EDCs in endometrial cancer [50]. A recent research project evaluated in a case-control study possible risk of **endometrial cancer** associated with exposure to environmental endocrine disruptors. The scientists analyzed the adipose tissue concentrations of polychlorinated biphenyls, hexachlorobenzene (HCB), p,p'-dichlorodiphenyl-dichloroethylene (p,p'-DDE), chlordanes, and polybrominated biphenyls in 76 cases with endometrial cancer and 39 controls with benign endometrial hyperplasia. The results suggested an interaction between p,p'-DDE and estrogen replacement drugs in the etiology of endometrial cancer, although no significant associations were found [104].

Temporal increases in the incidence of **testicular cancer** (TC) have been reported in certain countries, but rates vary considerably among countries. The risk started rising around 1910 in Nordic countries, and somewhat earlier in England and Wales, and therefore cannot be attributed solely to chemicals introduced in the mid or late twentieth century. Some evidence suggests that the incidence of cryptorchidism and hypospadias may show similar geographic variations to the incidence of testicular cancer and that these conditions may be developmentally linked. However, EDC exposure data for critical periods are lacking [50]. In 2003 a project reviewed 441 studies provided by a MEDLINE search using the key words testis/testicular, cancer/tumour and incidence that were published between 1980 and 2002.

From these articles they selected only those devoted to testis cancer incidence and of them only the most recent studies from each country or region. From these worldwide studies scientists observed a clear trend toward an increased **TC incidence** in the last 30 years in the majority of industrialized countries in North America, Europe and Oceania. Nevertheless, surprising differences in incidence rates were seen between neighbouring countries (Finland 2.5/100,000 cases versus Denmark 9.2/100,000) as well as among regions of the same country (2.8 to 7.9/100,000 according to various regional French registers). In addition, substantial differences in the TC incidence and trends were observed among ethnic groups. Scientists concluded that such a recent increase in the TC rate in most industrialized

countries should lead urologists and andrologists to give more attention to testicular cancer symptoms in adolescents and young adults, but identifying risk factors, especially EDCs, is very difficult [105].

Exposure to certain pesticides and organochlorines has been linked to increases in the incidence of **prostate cancer** in a few limited studies, but most studies have found no association, and the mechanism is unknown [50]. In the last 20 years epidemiological data and animal models suggested increasing evidence that specific EDCs may influence the development or progression of prostate cancer in humans. In large part, these effects appeared to be linked to interference with estrogen signaling, either through interacting with ERs or by influencing steroid metabolism and altering estrogen levels within the body. In humans, epidemiologic evidence links specific pesticides, PCBs and inorganic arsenic exposures to elevated prostate cancer risk. Studies in animal models also show augmentation of prostate carcinogenesis with several other environmental estrogenic compounds including cadmium, UV filters and BPA. Importantly, there appears to be heightened sensitivity of the prostate to these endocrine disruptors during the critical developmental windows including in utero and neonatal time points as well as during puberty. Thus infants and children may be considered a highly susceptible population for ED exposures and increased risk of prostate cancers with aging [106].

A direct association between exposure to EDCs and **thyroid cancer** has not been demonstrated. Overall, the biological plausibility of possible damage to certain human functions (particularly reproductive and developing systems) from exposure to EDCs seems strong when viewed against the background of known influences of endogenous and exogenous hormones on many of these processes. [50]. In a recent review, scientists studied the magnitude and uncertainties of ED chemicals, like Bisphenol A, phthalates and polybrominated ethers (that are used widely as plastic additives) to cause thyroid cancer. Theoretically, exposure and their effects on thyroid hormones for sensitive subpopulation groups like pregnant women, infants, and children can be possible. The reviewers found that the thyroid cancer risk gave qualitatively mixed associations [107].

14. Childhood Obesity and Endocrine Disruptors.

Prenatal exposure to several endocrine disruptors is associated with an elevated risk of overweight/obesity at later age, though this is not consistently reported in both genders. Dose-response relations appear not to be straight forward. Besides disruption of several hormonal pathways, including sex steroids and thyroid

hormones, and interference with PPARα and γ, prenatal exposure to EDCs could alter epigenetic control of gene expression, thus affecting developmental programming resulting in obesity. Scientific literature supports the hypothesis that prenatal exposure to EDCs may increase the risk of childhood obesity, but additional studies are needed to clarify dose-response relations as well as effects of mixed exposures [108].

A recent review reviewed the literature on the relations between exposure to chemicals with endocrine-disrupting abilities and obesity in humans. The studies generally indicated that exposure to some of the endocrine-disrupting chemicals was associated with an increase in body size in humans. The results depended on the type of chemical, exposure level, gender and timing of exposure. . The one study investigating relations with bisphenol A found no association. Studies investigating prenatal exposure indicated that exposure in utero may cause permanent physiological changes predisposing to later weight gain [109].

Another recent study proposed mechanisms that could underlie associations between EDCs and obesity, including effects on thyroid and steroid hormones, and activation of peroxisome proliferator-activated receptors, which play a major role in adipocyte differentiation and energy storage. Most evidence supporting the hypothesis that EDCs affect obesity comes from laboratory studies. Scientists summarize the limited epidemiological literature on the topic, including prospective studies of human prenatal exposure to EDCs [110].

Recent reviews on the EDCs and environmental risk to humans can be found in the scientific literature. Many substances from the technical and natural environment can cause damage to the endocrine system. Animal tests show that so-called EDCs, such as pesticides, fungicides, plasticizers (phthalates), bisphenol A (BPA), and organotin compounds can interfere with the endocrine system. In humans, it is difficult to attribute such changes to specific ED. Nevertheless, *in vitro* studies with human cells and tissues clearly show that EDCs are able to interfere with endogenous hormones. Several clinical studies show that humans are also affected, including reproductive disorders like reduction of spermatogenesis, decreased testosterone production or malformation of the genitals or induction of tumors like mammary carcinoma. Facing the body of reports documenting the effects of ED, the European Union supported--inter alia--COMPRENDO, a project addressing risk assessment of particular ED in human and wildlife species, while the FDA (Food and Drug Administration) supports the industry's actions to stop producing BPA-containing baby bottles and infant feeding cups. Further research is

needed to clarify whether the observed findings represent associations or causal results [111].

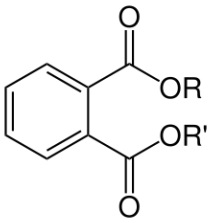
The **Endocrine Society** that was founded in 1916 is the world's oldest, largest, and most active organization devoted to research on hormones and the clinical practice of endocrinology. The Society works to foster a greater understanding of endocrinology amongst the general public and practitioners of complementary medical disciplines and to promote the interests of all endocrinologists at the national scientific research and health policy levels of government. In 2012 the Endocrine Society issued a **Statement of Principles** on EDCs:

".....The potential for deleterious effects of EDC must be considered relative to the regulation of hormone synthesis, secretion, and actions and the variability in regulation of these events across the life cycle. The developmental age at which EDC exposures occur is a critical consideration in understanding their effects. Because endocrine systems exhibit tissue-, cell-, and receptor-specific actions during the life cycle, EDC can produce complex, mosaic effects. This complexity causes difficulty when a static approach to toxicity through endocrine mechanisms driven by rigid guidelines is used to identify EDC and manage risk to human and wildlife populations. We propose that principles taken from fundamental endocrinology be employed to identify EDC and manage their risk to exposed populations. We emphasize the importance of developmental stage and, in particular, the realization that exposure to a presumptive "safe" dose of chemical may impact a life stage when there is normally no endogenous hormone exposure, thereby underscoring the potential for very low-dose EDC exposures to have potent and irreversible effects. Finally, with regard to the current program designed to detect putative EDC, namely, the **Endocrine Disruptor Screening Program**, we offer recommendations for strengthening this program through the incorporation of basic endocrine principles to promote further understanding of complex EDC effects, especially due to developmental exposures…." [112].

15. EDCs in Drinking Water and Consumer Products. The Case of Phthalates and Adverse Effects in Human Health

In the last 20 years, risk to human health at low levels of endocrine disrupting chemicals in drinking water has been hotly debated worldwide by scientists, government regulators, and consumer advocates. One particular EDC category that drew wide attention was phthalates which are found in our drinking water, air, and

food all over the world. Phthalates (plasticizers) are a major environmental pollutant and a cause for concern because they are found in most people's blood, tissue, breast milk, and urine [113].



General chemical structure of phthalates

Phthalates are a group of chemicals used to soften and increase the flexibility of plastic and vinyl. Phthalates are used in cosmetics and personal care products, including perfume, hair spray, soap, shampoo, etc. Phthalates are also used in wood finishes, detergents, adhesives, plastic plumbing pipes, lubricants, medical tubing and fluid bags, solvents, insecticides, medical devices, etc.

In the USA, current levels of seven phthalates studied by the **National Institute of Environmental Health Sciences** posed "minimal" concern for causing reproductive effects. But, high levels of exposure to di(2-ethylhexyl) phthalate through the use of medical tubing and other plastic devices for feeding, medicating, and assisting the breathing of newborn infants may affect the development of the male reproductive system, according to the National Institute of Environmental Health Sciences [http://toxtown.nlm.nih.gov/ text_version/chemicals.php?id=24].

The **European Union** banned phthalates in soft PVC toys and childcare products in 1999 through its Commission Decision 1999/815/EC. In 2004, the EU banned phthalates in cosmetics and other consumer products. The phthalates DEHP, BBP, and DBP (dibutyl phthalate) are restricted for all toys; DINP, DIDP (di-isodecyl phthalate) and DNOP are restricted only in toys that can be taken into the mouth. The restriction states that the amount of phthalates may not be greater than 0.1% mass percent of the plasticized part of the toy. These phthalates are allowed at any concentration in other products and other phthalates are not restricted. There are no other specific restrictions in the European Union, although draft proposals have been tabled for the inclusion of BBP, DEHP, and DBP on the Candidate list of Substances for Authorisation under REACH [114].

The **U.S. Food and Drug Administration** (FDA) stated that there is no clear evidence of harm from phthalates in cosmetics and other products. On October 19, 2011, the Food and Drug Administration (FDA) published a final rule in the Federal Register (76 FR 64810) that amended its bottled water standard of quality regulations

by establishing an allowable level for di(2-ethylhexylphthalate) (DEHP). The final rule is effective on April 16, 2012. As a consequence, bottled water manufacturers are required to monitor their finished bottled water products for DEHP as often as necessary, but at least once each year under the current good manufacturing practice (CGMP) regulations for bottled water [http://www.fda.gov/Food/ GuidanceComplianceRegulatoryInformation/…..].

Reviews on public health risk from phthalates in drinking water and consumer products showed that there is limited risk for adverse health effects at low concentrations in drinking water (mostly contaminations of migration from packaging). Analysis of all of the available data leads to the conclusion that the risks are low, even lower than originally thought, and that there is no convincing evidence of adverse effects to humans. Since the scientific evidence strongly suggests that risks to humans are low, phthalate regulations that have been enacted are unlikely to lead to any marked improvement in public health [115, 116].

16. Endocrine-Disrupters Chemicals in Food and Packaging. Bisphenol A a Special Case of Plasticizer

The plastics manufacturers in order to improve the quality and properties of their products use various additives (for example, plasticizers, stabilizers and antioxidants). The polymers used for packaging are mainly polystyrene, PS], polycarbonate [(water bottles), PC], polyvinyl chloride, (PVC), polyethylene products with different molecular weights and PET or Polyethylene terephthalate which is a thermoplastic polymer resin used in synthetic fibers; beverage, food and other liquid containers.

Some of the plasticizers used in these polymers phthalates. Inevitably the concern for endocrine disrupting properties and adverse effects caused great concern among consumers. There were many demands for banning these plasticizers. Some studies showed increased estrogenicity in foods that were packaged in these polymeric materials. The results of these studies were controversial for many years, while the consumers were bombarded with extremely negative suggestions. Some manufacturers in order to avoid negative demands of their products and reduce the anxiety of parents, especially for soft toys from PVC, removed completely the phthalates from their products.

Figure 10. Bisphenol A as a plasticizer in polymers has been replaced because of negative consumer perception despite the indications by many studies that is safe.

The **European Union** (EU) put restriction on the marketing and use of certain Phathalates. The 2005. Directive 2005/84/EC of the European Parliament and the Council for the 22nd time Council Directive 76/769/EEC was enacted on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances (**phthalates in toys and childcare articles**).OJL344, 27.12.2005[http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:en:PD].

A special case which until now was in the centre of this controversy was **Bisphenol A [BPA]** for polycarbonate polymers, used mainly for food packaging. BPA was found as contaminant in food and consumer products at varied concentrations depending on physicochemical conditions such as temperature, UV light, pH, microwave, and mechanical stress. Some phthalates (for example, DEHP, DBP) and Bisphenol have been suspected to have endocrine disrupting effects, but human toxicological effects of these compounds are very controversial.

For these reasons, a comprehensive review on toxicological and risk assessment studies for these chemicals was carried out to evaluate their safety in humans. On the basis of exposure estimates for these chemicals scientists developed various toxicological standards. One is the reference doses (RfDs) which calculate the hazard index (HI = chronic daily intake/tolerable daily intake [TDI] or RfD). An index of HI of less than 1 suggests an exposure lower than the safety limit of the chemicals. Studies showed that HI of these chemicals were lower then 1, but there were several exceptions for DEHP, DBP), DIDP), and DnOP. This review suggested that the use of plastic food containers might not exceed human safe limits

in general with respect to endocrine disruptors aside from the exceptions of the phthalates mentioned earlier [117].

A recent biomonitoring study of DEHA was contacted with German infants. The study was conducted with a population of 25 German subjects aged between 15 and 21 months. Overall, 16 phthalates and DEHA were measured by GC-MS in a total of 171 duplicate diet samples collected over 7 days. 20 phthalate metabolites were analyzed in the urine samples by LC-MS/MS. collected over 7 consecutive days. The comparison of the two intake estimates indicates that the dominant intake source of DEHP was food ingestion, whereas other sources considerably contributed to the total intake of other phthalates. Using our “high” intake scenario, none of the analyzed phthalates reached the recommended tolerable daily intake levels [118].

A recent review on Bisphenol A summarized the great number of studies and the controversy caused. More than 5000 safety-related studies have been published on Bisphenol A (BPA), there seems to be no resolution of the apparently deadlocked controversy as to whether exposure of the general population to BPA causes adverse effects due to its estrogenicity. Therefore, the **Advisory Committee of the German Society of Toxicology** reviewed the background and cutting-edge topics of this BPA controversy. The current tolerable daily intake value (TDI) of 0.05 mg/kg body weight [bw]/day, derived by the **European Food Safety Authority (EFSA)**, is mainly based on body weight changes in two- and three-generation studies in mice and rats. Recently, these studies and the derivation of the TDI have been criticized. The ACGST Committee came to the conclusion that rodent data can well be used as a basis for human risk evaluation. Data from toxicokinetics studies show that the half-life of BPA in adult human subjects is less than 2 hours and BPA is completely recovered in urine as BPA-conjugates. Biomonitoring studies that have been used to estimate human BPA exposure showed that the daily intake of BPA is far below the TDI for the general population. Overall, the ACGST Committee concluded that the current TDI for BPA is adequately justified and that the available evidence indicates that BPA exposure represents no noteworthy risk to the health of the human population, including newborns and babies. [119].

A recent review concentrated to evaluate the contribution of food and non-food sources to the human exposure to Bisphenol A. Based on the available data for these exposure sources, it was concluded that the exposure to BPA from non-food sources is generally lower than that from exposure from food by at least one order of magnitude for most studied subgroups. The use of urinary concentrations from biomonitoring studies was evaluated and the back-calculation of BPA intake seems

reliable for the overall exposure assessment. In general, the total exposure to BPA is several orders of magnitude lower than the current tolerable daily intake [TDI] of 50 μg/kg bw/day. [120].

17. Occurrence and Fate of Endocrine Disrupter Chemicals in Environmental Measurements from Greece

Greek scientists studied in the past years the presence of endocrine disrupting chemicals in sewage treatment plant and in inland waters in various parts of Greece. We collected some of the most important papers:

1. Stasinakis AS, Gatidou G, Mamais D, Thomaidis NS, Lekkas TD. Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. *Water Res* 42:1796-1804, 2008.
2. Maragou NC, Makri A, Lambi EN, Thomaidis NS, Koupparis MA.. Migration of bisphenol A from polycarbonate baby bottles under real use conditions. *Food Addit Conatmin:Part A* 25(3):373-383, 2008.
3. Pothitou P, Voutsa D. Endocrine disrupting compounds in municipal and industrial wastewater treatment plants in Northern Greece. *Chemosphere* 73:1716-1723, 2008.
4. Arditsoglou A, Voutsa D. Partitioning of endocrine disrupting compounds in island waters and wastewater discharged into the central areas of Thessaloniki, Northern Greece. *Environ Sci Pollut Res* 17: 529-538, 2010.
5. Gatidou G**,** Vassalou E, Thomaidis NS. Bioconcentration of selected endocrine disrupting compounds in the Mediterranean mussel, *Mytilus galloprovincialis. Marin Pollut Bull* 60(11): 2111-2116, 2010.
6. Stasinakis AS, Kordoulis CI, Tsiouma V, Gatidou G, Thomaidis NS. Removal of selected endocrine disrupters in activated sludge systems: Effects of sludge retention time on their sorption and biodegradation. *Bioresource Technol* 101(7):2090-2095, 2010.
7. Salapasidou M, Samara C, Voutsa D. Endocrine disrupting compounds in the atmosphere of the urban area of Thessaloniki, Greece. *Atmosph Environ* 45(22): 3720-3729, 2011.
8. Samaras VG, Thomaidis NS, Stasinakis AS. An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endoccrine disrupting chemicals in wastewater and sewage. Anal Bioanal Chem 399:2549-2561, 2011.
9. Samaras VG, Farmaki E, Thomaiidis NS. Occurrence of endocrine disrupters and selected pharmaceuticals in Aisonas River (Greece) and environmental risk assessment using hazard indexes. *Environ Sci Pollut Res* (on line) 2011 (DOI:10.1007/s11356-0661-7)

18. Concluding Remarks on Endocrine Disrupting Chemicals Assessment Reports

In 2012 a group of scientists wrote a critique of the European Commission Document :State of the Art Assessment of Endocrine Disrupters" [Rhomberg LR, Goodman JE, Foster WG, Borgert CJ, Van Der Kraak G. "A Critique of the European Commission Document :**State of the Art Assessment of Endocrine Disrupters". *Critical Reviews in Toxicology* 42(6): 465-473, 2012**]. This critique shoes that despite all these years of research and efforts to establish a structured risk assessment and adverse health effects of EDCs there are many different opinions among scientists.

The critique emphasized "…. *The 2002 WHO/IPCS document [50] was generated over several years in a very structured manner. First, IPCS and the OECD convened an informal consultation in 1997. A Steering Group of scientific experts was convened and met seven times over three years to provide oversight, expertise, and guidance for the project and to evaluate the accuracy, significance, and relevance of the information in the document. A preliminary draft of the document was circulated to several additional scientific experts and IPCS contact points for review. In total, dozens of international scientific experts contributed to the WHO/IPCS document. This report has become a guiding document in ED, as shown by more than 260 citations since its publication in 2002…."*

"…*More recently, in January 2012, the European Union (EU) Directorate-General for the Environment (DG Environment) finalized and posted on the internet a separate "**State of the Art Assessment of Endocrine Disrupters**" (henceforth called the "SOA Assessment"), which had been commissioned in 2009 to provide a basis for developing scientific criteria for identifying endocrine disrupting chemicals (EDCs) and reviewing and possibly revising the European Community Strategy on Endocrine Disrupters The stated objectives of the study were to "(i) review the scientific knowledge published in the literature over the last 10 years and in the reports of more than 80 [EU] funded projects; (ii) review the approaches for assessment of endocrine disrupters used in selected Member States, in major competing economies outside the EU and in international bodies; and (iii) draw conclusions and answer policy relevant questions"). Although this report has been produced under a contract from the DG Environment, and has no connection to WHO/IPCS, it is clearly intended for* the DG Environment's use as a successor to and update of the 2002 WHO/IPCS evaluation.

"… *Although the SOA Assessment is completely independent of the 2002 WHO/IPCS report, it clearly used the 2002 report as a baseline, aiming to extend the earlier evaluations with new information. The 2002 report included an evaluation of all relevant primary studies in the fields of reproductive/developmental and endocrine toxicology and underwent extensive planning and peer review. In contrast, the SOA Assessment is a self-described "review of reviews" that does not include a complete evaluation of individual studies. The number of studies increased substantially in the last decade with conflicting results.* ***In Conclusions the authors stated "….*** *As we noted at the outset of these comments, considerable attention has been focused on the potential for ED by exposure to exogenous chemicals since the WHO/IPCS review of 2002, and we applaud the resolution of the DG Environment to establish an up-to-date basis for its further policy decisions. Moving forward, sound policies must take account of this growing area of environmental science and should be based on a full understanding of all the available information, including its strengths and shortcomings, variations, inconsistencies, and outstanding questions. We recognize the challenge of accomplishing this in a single review. In our view, however, the SOA Assessment should be seen as a start that currently falls well short of what will be needed. It raises some issues and notes some published observations that will be relevant in addressing them, but it lacks a systematic evaluation of the literature and a rigorous basis for bringing that literature to bear on the key questions. It lacks a systematic and transparent method for selecting the studies to be included in the review, does not identify the specific literature that was reviewed, and appears to have overlooked important and significant literature critical to a balanced review. It does not note strengths and weaknesses of individual studies that ought to bear on their interpretation, and it fails to assess whether findings across studies addressing the same chemicals or endpoints find consistent results. ED is a set of modes of action, rather than a set of adverse outcome results, yet the SOA Assessment does not integrate consideration of dose-response or the underlying sciences of endocrinology and pharmacology into its evaluations. It follows no clear WOE methodology in its assessment of the interpretation of existing studies and thereby fails to support its conclusions adequately. The failure to address the evidence and reasons behind changes in conclusions vis-à-vis the earlier 2002 WHO/IPCS review is especially concerning. A number of notable and highly visible scientific debates that are current in the field are not characterized or in some cases even noted, though* the spectrum of opinion and the evidence adduced to support different views are undeniably a part of the "state of the science……."

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