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State of the Science of Endocrine Disrupting Chemicals - 2012

Edited by
Åke Bergman, Jerrold J. Heindel, Susan Jobling,
Karen A. Kidd and R. Thomas Zoeller

IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

State of the Science of

Endocrine Disrupting Chemicals - 2012

An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization.

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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Preface

The *State of the Science of Endocrine Disrupting Chemicals—2012*, is an update of the scientific knowledge, including main conclusions and key concerns, on endocrine disruptors as part of the ongoing collaboration between the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) to address concerns about the potential adverse health effects of chemicals on humans and wildlife.

We live in a world in which man-made chemicals have become a part of everyday life. It is clear that some of these chemical pollutants can affect the endocrine (hormonal) system, and certain of these endocrine disruptors may also interfere with the developmental processes of humans and wildlife species. Following international recommendations in 1997 by the Intergovernmental Forum on Chemical Safety and the Environment Leaders of the Eight regarding the issue of endocrine disrupting chemicals (EDCs), WHO, through the International Programme on Chemical Safety (IPCS), a joint programme of WHO, UNEP and the International Labour Organization, developed in 2002 a report entitled *Global Assessment of the State-of-the-Science of Endocrine Disruptors.*

The Strategic Approach to International Chemicals Management (SAICM) was established by the International Conference on Chemicals Management (ICCM) in February 2006, with the overall objective to achieve the sound management of chemicals throughout their life cycle so that, by 2020, chemicals are used and produced in ways that minimize significant adverse effects on human health and the environment.

SAICM recognizes that risk reduction measures need to be improved to prevent the adverse effects of chemicals on the health of children, pregnant women, fertile populations, the elderly, the poor, workers and other vulnerable groups and susceptible environments. It states that one measure to safeguard the health of women and children is the minimization of chemical exposures before conception and through gestation, infancy, childhood and adolescence.

SAICM also specifies that groups of chemicals that might be prioritized for assessment and related studies, such as for the development and use of safe and effective alternatives, include chemicals that adversely affect, inter alia, the reproductive, endocrine, immune or nervous systems. A resolution to include EDCs as an emerging issue under SAICM was adopted in September 2012 by ICCM at its third session.

EDCs represent a challenge, as their effects depend on both the level and timing of exposure, being especially critical when exposure occurs during development. They have diverse applications, such as pesticides, flame retardants in different products, plastic additives and cosmetics, which may result in residues or contaminants in food and other products. Therefore, EDCs may be released from the products that contain them.

The protection of the most vulnerable populations from environmental threats is a key component of the Millennium Development Goals. As the challenge in meeting the existing goals increases, with work under way in developing countries to overcome traditional environmental threats while dealing with poverty, malnutrition and infectious disease, emerging issues should be prevented from becoming future traditional environmental threats. Endocrine disruption is a challenge that must continue to be addressed in ways that take into account advances in our knowledge.

UNEP and WHO, in collaboration with a working group of international experts, are taking a step forward by developing these documents on endocrine disruptors, including scientific information on their impacts on human and wildlife health and key concerns for decision-makers and others concerned. The well-being of future human and wildlife generations depends on safe environments.

UNEP and WHO convened, in December 2009, a meeting of the planning group for the development of an update to the 2002 IPCS "Global Assessment of the State-of-the-Science of Endocrine Disruptors". This was followed by teleconferences and a planning meeting

in Geneva in June 2010. These meetings allowed for defining the scope, the outline, the development process and suggestions of main authors that would be integrated in the working group. Authors were identified because of previous peer-reviewed publications and according to their area of expertise. The following experts provided guidance and expertise for the planning stages:

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- Niels Erik Skakkebaek, University of Copenhagen, Denmark
- Hans-Christian Stolzenberg, Federal Environment Agency, Germany
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The working group consequently met in Stockholm in November 2010, in Copenhagen in May 2011 and in Geneva in December 2011, as well as through teleconferences, to develop and revise various drafts of the documents. Professor Åke Bergman led the working group and facilitated the development of the chapters with the main authors in coordination with UNEP and WHO.

The following international scientific experts were part of the working group that developed the documents:

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The development of these documents would not have been made possible without the significant contributions of the planning and working groups and the valuable leadership of Professor Åke Bergman, as well as of the lead authors of the main chapters Professor Susan Jobling, Dr. Jerrold J. Heindel, Professor Karen A. Kidd and Professor R. Thomas Zoeller. UNEP and WHO are very grateful for their extensive support and for the hard work of all.

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The working group members, scientific experts and contributors of text served as individual scientists and not as representatives of any organization, government or industry. All individuals who participated in the preparation of these documents served in their personal capacity and were required to sign a Declaration of Interest statement informing the Responsible Officer if, at any time, there was a conflict of interest perceived in their work. Such a procedure was followed, and no conflicts of interest were identified.

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Executive summary

Introduction

In 2002, the International Programme on Chemical Safety (IPCS), a joint programme of the World Health Organization (WHO), the United Nations Environment Programme (UNEP) and the International Labour Organization, published a document entitled *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002). This work concluded that scientific knowledge at that time provided evidence that certain effects observed in wildlife can be attributed to chemicals that function as endocrine disrupting chemicals (EDCs); that the evidence of a causal link was weak in most cases and that most effects had been observed in areas where chemical contamination was high; and that experimental data supported this conclusion. The document further concluded that there was only weak evidence for endocrine-related effects in humans. Uncertainties regarding global endocrine disrupting effects were put forward; simultaneously, concern was expressed that endocrine disruption may affect developmental processes if exposure occurs during early life stages. Almost no data regarding endocrine-related effects were available for chemicals other than those defined as persistent organic pollutants (POPs) according to the Stockholm Convention on Persistent Organic Pollutants: polychlorinated biphenyls (PCBs), dioxins and dichlorodiphenyltrichloroethane (DDT). Even for these chemicals, the data gaps were obvious for parts of the world other than western Europe, North America and Japan. The IPCS (2002) document finally concluded that there was a need for broad, collaborative and international research initiatives and presented a list of research needs.

Since the start of this century, intensive scientific work has improved our understanding of the impacts of EDCs on human and wildlife health. Scientific reviews published by, for example, the Endocrine Society (Diamanti-Kandarakis et al., 2009), the European Commission (Kortenkamp et al., 2011) and the European Environment Agency (2012) show the scientific complexity of this issue. These documents implicate EDCs as a concern to public and wildlife health. In addition, the European Society for Paediatric Endocrinology and the Pediatric Endocrine Society have put forward a consensus statement calling for action regarding endocrine disruptors and their effects (Skakkebaek et al., 2011).

Now, in 2012, the United Nations Environment Programme (UNEP) and WHO present an update of the IPCS (2002) document, entitled *State of the Science of Endocrine Disrupting Chemicals—2012*. This document provides the global status of scientific knowledge on exposure to and effects of EDCs. It explains, in the first chapter, what endocrine disruption is all about, and then it discusses in detail, in 12 sections in the second chapter, endocrine disrupting effects in humans and wildlife. The work is based on the fact that endocrine systems are very similar across vertebrate species and that endocrine effects manifest themselves independently of species. The effects are endocrine system related and not necessarily species dependent. Effects shown in wildlife or experimental animals may also occur in humans if they are exposed to EDCs at a vulnerable time and at concentrations leading to alterations of endocrine regulation. Of special concern are effects on early development of both humans and wildlife, as these effects are often irreversible and may not become evident until later in life. The third and final chapter of this document discusses exposure of humans and wildlife to EDCs and potential EDCs.

Key concerns

- **Human and wildlife health depends on the ability to reproduce and develop normally. This is not possible without a healthy endocrine system.**
- **Three strands of evidence fuel concerns over endocrine disruptors:**
  - the high incidence and the increasing trends of many endocrine-related disorders in humans;
  - observations of endocrine-related effects in wildlife populations;

- the identification of chemicals with endocrine disrupting properties linked to disease outcomes in laboratory studies.

- **Many endocrine-related diseases and disorders are on the rise.**
  - Large proportions (up to 40%) of young men in some countries have low semen quality, which reduces their ability to father children.
  - The incidence of genital malformations, such as non-descending testes (cryptorchidisms) and penile malformations (hypospadias), in baby boys has increased over time or levelled off at unfavourably high rates.
  - The incidence of adverse pregnancy outcomes, such as preterm birth and low birth weight, has increased in many countries.
  - Neurobehavioural disorders associated with thyroid disruption affect a high proportion of children in some countries and have increased over past decades.
  - Global rates of endocrine-related cancers (breast, endometrial, ovarian, prostate, testicular and thyroid) have been increasing over the past 40–50 years.
  - There is a trend towards earlier onset of breast development in young girls in all countries where this has been studied. This is a risk factor for breast cancer.
  - The prevalence of obesity and type 2 diabetes has dramatically increased worldwide over the last 40 years. WHO estimates that 1.5 billion adults worldwide are overweight or obese and that the number with type 2 diabetes increased from 153 million to 347 million between 1980 and 2008.

- **Close to 800 chemicals are known or suspected to be capable of interfering with hormone receptors, hormone synthesis or hormone conversion. However, only a small fraction of these chemicals have been investigated in tests capable of identifying overt endocrine effects in intact organisms.**
  - The vast majority of chemicals in current commercial use have not been tested at all.
  - This lack of data introduces significant uncertainties about the true extent of risks from chemicals that potentially could disrupt the endocrine system.

- **Human and wildlife populations all over the world are exposed to EDCs.**
  - There is global transport of many known and potential EDCs through natural processes as well as through commerce, leading to worldwide exposure.
  - Unlike 10 years ago, we now know that humans and wildlife are exposed to far more EDCs than just those that are POPs.
  - Levels of some newer POPs in humans and wildlife are still increasing, and there is also exposure to less persistent and less bioaccumulative, but ubiquitous, chemicals.
  - New sources of human exposure to EDCs and potential EDCs, in addition to food and drinking-water, have been identified.
  - Children can have higher exposures to chemicals compared with adults—for example, through their hand-to-mouth activity and higher metabolic rate.

- **The speed with which the increases in disease incidence have occurred in recent decades rules out genetic factors as the sole plausible explanation. Environmental and other non-genetic factors, including nutrition, age of mother, viral diseases and chemical exposures, are also at play, but are difficult to identify. Despite these difficulties, some associations have become apparent:**
  - Non-descended testes in young boys are linked with exposure to diethylstilbestrol (DES) and polybrominated diphenyl ethers (PBDEs) and with occupational pesticide exposure during pregnancy. Recent evidence also shows links with the pain-killer paracetamol. However, there is little to suggest that PCBs or dichlorodiphenyldichloroethylene (DDE) and DDT are associated with cryptorchidism.
  - High exposures to polychlorinated dioxins and certain PCBs (in women who lack some detoxifying enzymes) are risk factors in breast cancer. Although exposure to natural and synthetic estrogens is associated with breast cancer, similar evidence linking estrogenic environmental chemicals with the disease is not available.
  - Prostate cancer risks are related to occupational exposures to pesticides (of an unidentified nature), to some PCBs and to arsenic. Cadmium exposure has been linked with prostate cancer in some, but not all, epidemiological studies, although the associations are weak.
  - Developmental neurotoxicity with negative impacts on brain development is linked with PCBs. Attention deficit/hyperactivity disorder (ADHD) is over-represented in populations with elevated exposure to organophosphate pesticides. Other chemicals have not been investigated.
  - An excess risk of thyroid cancer was observed among pesticide applicators and their wives, al-

though the nature of the pesticides involved was not defined.

- **Significant knowledge gaps exist as to associations between exposures to EDCs and other endocrine diseases, as follows:**
  - There is very little epidemiological evidence to link EDC exposure with adverse pregnancy outcomes, early onset of breast development, obesity or diabetes.
  - There is almost no information about associations between EDC exposure and endometrial or ovarian cancer.
  - High accidental exposures to PCBs during fetal development or to dioxins in childhood increase the risk of reduced semen quality in adulthood. With the exception of these studies, there are no data sets that include information about fetal EDC exposures and adult measures of semen quality.
  - No studies exist that explore the potential link between fetal exposure to EDCs and the risk of testicular cancer occurring 20–40 years later.
- **Numerous laboratory studies support the idea that chemical exposures contribute to endocrine disorders in humans and wildlife. The most sensitive window of exposure to EDCs is during critical periods of development, such as during fetal development and puberty.**
  - Developmental exposures can cause changes that, while not evident as birth defects, can induce permanent changes that lead to increased incidence of diseases throughout life.
  - These insights from endocrine disruptor research in animals have an impact on current practice in toxicological testing and screening. Instead of solely studying effects of exposures in adulthood, the effects of exposures during sensitive windows in fetal development, perinatal life, childhood and puberty require careful scrutiny.
- **Worldwide, there has been a failure to adequately address the underlying environmental causes of trends in endocrine diseases and disorders.**
  - Health-care systems do not have mechanisms in place to address the contribution of environmental risk factors to endocrine disorders. The benefits that can be reaped by adopting primary preventive measures for dealing with these diseases and disorders have remained largely unrealized.
- **Wildlife populations have been affected by endocrine disruption, with negative impacts on growth and reproduction. These effects are widespread and have been due primarily to POPs. Bans of these chemicals have reduced exposure and led to recovery of some populations.**
  - It is therefore plausible that additional EDCs, which have been increasing in the environment and are of recent concern, are contributing to current population declines in wildlife species. Wildlife populations that are also challenged by other environmental stressors are particularly vulnerable to EDC exposures.
- **Internationally agreed and validated test methods for the identification of endocrine disruptors capture only a limited range of the known spectrum of endocrine disrupting effects. This increases the likelihood that harmful effects in humans and wildlife are being overlooked.**
  - For many endocrine disrupting effects, agreed and validated test methods do not exist, although scientific tools and laboratory methods are available.
  - For a large range of human health effects, such as female reproductive disorders and hormonal cancers, there are no viable laboratory models. This seriously hampers progress in understanding the full scale of risks.
- **Disease risk due to EDCs may be significantly underestimated.**
  - A focus on linking one EDC to one disease severely underestimates the disease risk from mixtures of EDCs. We know that humans and wildlife are simultaneously exposed to many EDCs; thus, the measurement of the linkage between exposure to mixtures of EDCs and disease or dysfunction is more physiologically relevant. In addition, it is likely that exposure to a single EDC may cause disease syndromes or multiple diseases, an area that has not been adequately studied.
- **An important focus should be on reducing exposures by a variety of mechanisms. Government actions to reduce exposures, while limited, have proven to be effective in specific cases (e.g. bans and restrictions on lead, chlorpyrifos, tributyltin, PCBs and some other POPs). This has contributed to decreases in the frequency of disorders in humans and wildlife.**
- **Despite substantial advances in our understanding of EDCs, uncertainties and knowledge gaps still exist that are too important to ignore. These knowledge gaps hamper progress towards better protection of the public and wildlife. An integrated, coordinated international effort is needed to define the role of EDCs in current declines in human and wildlife health and in wildlife populations.**

General aspects on endocrine disruption (chapter 1)

The present document uses the same definitions of EDCs and potential EDCs that were developed in IPCS (2002): "An *endocrine disruptor* is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations"; and "A *potential endocrine disruptor* is an exogenous substance or mixture that possesses properties that might be expressed to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations".

In addition to the key concerns presented above, the most relevant main messages from chapter 1 are presented below:

- What is endocrine disruption all about? Some endocrine disruptors can act directly on hormone receptors as hormone mimics or antagonists. Others can act directly on any number of proteins that control the delivery of a hormone to its normal target cell or tissue.
- The affinity of an endocrine disruptor to a hormone receptor is not equivalent to its potency. Chemical potency on a hormone system is dependent upon many factors.
- Endocrine disruptors produce non-linear dose–response curves both in vitro and in vivo, by a variety of mechanisms.
- Environmental chemicals can exert endocrine disrupting activity on more than just estrogen, androgen and thyroid hormone action. Some are known to interact with multiple hormone receptors simultaneously.
- Sensitivity to endocrine disruption is highest during tissue development; developmental effects will occur at lower doses than are required for effects in adults.
- Testing for endocrine disruption must encompass the developmental period and include lifelong follow-up to assess latent effects.
- Endocrine disruption represents a special form of toxicity, and this must be taken into consideration when interpreting the results of studies on EDCs or when designing studies to clarify the effects of EDCs and quantifying the risks to human and wildlife health.

Over the last 10 years, it has been established that endocrine disruptors can work together to produce additive effects, even when combined at low doses that individually do not produce observable effects.

Evidence for endocrine disruption in humans and wildlife (chapter 2)

Over the last decade, scientific understanding of the relationship between exposure to endocrine disruptors and health has advanced rapidly. There is a growing concern that maternal, fetal and childhood exposure to EDCs could play a larger role in the causation of many endocrine diseases and disorders than previously believed. This is supported by studies of wildlife populations and of laboratory animals showing associations between exposure to EDCs and adverse health effects and by the fact that the increased incidence and prevalence of several endocrine disorders cannot be explained by genetic factors alone. Epidemiological studies to date have explored quite narrow hypotheses about a few priority pollutants, without taking account of combined exposures to a broader range of pollutants. The main messages for each endocrine disease or disorder described in chapter 2 are presented below, focusing on advances in knowledge and understanding since publication of the IPCS (2002) report.

Female reproductive health

- Increased understanding of endocrine pathways governing female reproductive processes suggests that a role for EDCs in the multicausality of female reproductive dysfunction is biologically plausible.
- There is limited and conflicting experimental and epidemiological evidence to support a role for EDCs in advancing puberty and breast development and in causing fibroids (phthalates) and endometriosis (PCBs, phthalates and dioxins) and almost no evidence for causation of polycystic ovary syndrome or infertility; however, few studies have examined chemical causation of these diseases directly, and very few chemicals have been investigated.
- Historically high incidences of fibroids have also occurred in seal populations in the Baltic Sea and have been associated with exposure to contaminants (particularly PCBs and organochlorine pesticides). Recovery of these populations is now occurring, following a decline in the environmental concentrations of these chemicals. More evidence now exists that reduced reproductive success in female birds, fish and gastropods is related to exposure to PCBs, organochlorine pesticides, tributyltin and dioxins. As exposure to these EDCs decreased, adverse reproductive effects in wild populations also decreased.
- There is more evidence from laboratory studies now than in 2002 that chemical exposures can interfere with endocrine signalling of pubertal timing, fecundity and fertility and with menopause.

- There are many gaps in our knowledge of endocrine disruption of the female reproductive system. Many of the mechanisms are poorly understood, and the number of chemicals that have been investigated is limited.
- There are many gaps in the available chemical test methods for screening chemicals for endocrine disrupting effects on female reproduction. Regulatory tests for many wildlife taxa are currently not developed, and the endocrine end-points measured in mammalian assays are sometimes not adequate to detect possible roles of EDCs in inducing many of the female reproductive disorders and diseases described here.

Male reproductive health

- In comparison with 2002, the incidence of testicular cancer has further increased in the European countries in which it has been carefully studied.
- Although geographical differences exist, semen quality has declined in some countries; 20–40% of young men in the general population of Denmark, Finland, Germany, Norway and Sweden have sperm counts in the subfertile range.
- Decreases in semen quality reported in Scandinavian studies parallel increases in the incidence of both genital abnormalities in babies and testis germ cell cancer in men in the same areas over the last 60 years. The occurrence of cryptorchidism at birth is associated with a 5-fold increased risk of testicular cancer and with impaired semen quality and subfecundity.
- Several epidemiological studies show weak associations between cryptorchidism in sons and exposure of their mothers to DES, paracetamol, mixtures of PBDEs or unknown pesticides during pesticide application. No associations have been found with individual pesticides, underlining the importance of including mixtures assessment in epidemiological and laboratory investigations. Studies have not identified associations with PCBs or with DDT/DDE.
- High accidental exposures to PCBs during fetal development or to dioxins in childhood increase the risk of reduced semen quality in adulthood. With the exception of these studies, there are no data sets that include information about fetal EDC exposures and adult measures of semen quality. No studies have been performed to explore the potential link between fetal EDCs and the risk of testicular cancer occurring 20–40 years later.
- Limited evidence suggests a slightly increased risk of hypospadias or of reduced semen quality associated with exposure to mixtures of endocrine disrupting pesticides. Limited evidence also suggests links between maternal phthalate exposure and reduced anogenital distance (a proxy for reduced semen quality) in baby boys. For most chemicals, potential associations between fetal exposure and childhood or adult male reproductive health have not been studied.
- An animal model for aspects of testicular dysgenesis syndrome has been established in the rat and shows an interrelationship between testicular dysgenesis and exposure to some EDCs during the fetal male programming window. There is now a mechanism demonstrated in the rat by which irreversible disorders of the male reproductive tract can be caused.
- Exposures to several anti-androgenic pesticides have been shown to induce cryptorchidism, hypospadias and reduced semen quality in rodent experiments and are also often linked to shortened anogenital distance.
- Not all effects seen in the rat appear across species, and vice versa. Recent data show that effects of phthalates in the rat are not seen in the mouse or in human testis studied in culture. For bisphenol A (BPA), the human testis model is more sensitive to toxic effects than the rat model.
- With the exception of testicular germ cell cancers, which are logistically difficult to detect, symptoms of androgen deficiency and estrogen exposure occur in a variety of wildlife species in both urban and rural environments and have been linked to exposure to chemicals in a limited number of species in some areas.
- The feminizing effects of estrogenic chemicals from sewage effluents on male fish were first reported in the 1990s and have now been seen in many countries and in several species of fish, indicating that this is a widespread phenomenon. Feminized (intersex) male fish have reduced sperm production and reduced reproductive success.
- The suite of effects seen in wildlife can be reproduced in laboratory studies in which experimental animals are exposed to EDCs.

Sex ratio

- EDC-related sex ratio imbalances, resulting in fewer male offspring in humans, do exist (e.g. in relation to dioxin and 1,2-dibromo-3-chloropropane), although the underlying mechanisms are unknown. The effects of dioxin on sex ratio are now corroborated by results obtained in the mouse model.
- EDC-related sex ratio imbalances have been seen in wild fish and molluscs, and the effects of EDCs on sex ratios in some of these species are also supported by laboratory evidence.

Thyroid-related disorders

- Compared with 2002, increased but still limited evidence exists showing associations between thyroid-related disorders and chemical exposures. There is, however, very little direct evidence that effects on thyroid hormone action mediate these associations. There is currently no direct approach to test this hypothesis on human populations.
- Some epidemiological studies report associations between chemical exposures (PCBs, PBDEs, phthalates, BPA and perfluorinated chemicals) and thyroid function, including in pregnant women, but few of these report associations with thyroid measures in the cord blood of their offspring or with abnormal function in these offspring.
- Laboratory experiments with rodents show that there are many chemicals that can interfere with thyroid function. For example, exposure to PCBs clearly reduces serum thyroid hormone levels in rodents.
- Similarly, there are chemicals that can interfere directly with thyroid hormone action in a manner that will not be captured by measuring serum hormone levels only.
- The variability of effects seen is interpreted by some to indicate that there is no convincing evidence that chemicals can interfere with thyroid hormone action in humans.
- Evidence of relationships between exposure to chemicals and thyroid hormone disruption in wildlife species has increased in the last decade, especially in relation to exposure to the flame retardant PBDEs and PCBs, but other chemicals are inadequately studied.
- The strength of evidence supporting a role for EDCs in disrupting thyroid function in wildlife adds credence to the hypothesis that this could occur in humans.
- Thyroid disruption is acknowledged to be poorly addressed by the chemical tests currently listed in the Organisation for Economic Co-operation and Development conceptual framework. Genetic lines of mice are now widely available that could help clarify the mechanisms by which chemical exposures can interfere with thyroid hormone action.

Neurodevelopmental disorders in children and wildlife

- There are some strong data sets (e.g. for PCBs, lead and methylmercury) showing that environmentally relevant developmental exposures to these EDCs and potential EDCs have caused cognitive and behavioural deficits in humans.
- Sufficient data indicate that in utero exposure to EDCs also affects cognition in animal studies, and limited data indicate that sexually dimorphic behaviours are also affected.
- Studies of exposed wildlife provide important information on exposure levels, early and subclinical effects and the clinical neurotoxicity of EDCs, because the mechanisms, underlying effects and outcomes of exposures are often similar to those in humans. Data showing effects on growth, development and behaviour in wildlife exist for some PCBs and mercury, but are sparse or non-existent for other EDCs.
- Since 2002, increased evidence supports the involvement of thyroid hormone mechanisms in neurodevelopmental disorders in humans and wildlife and the sensitivity of embryonic and postnatal development to EDCs when compared with adulthood.
- Severe thyroid hormone deficiency causes severe brain damage. Moderate (25%) or even transient insufficiency of thyroxine during pregnancy is also associated with reduced intelligence quotient, ADHD and even autism in children.
- Chemical testing strategies do not routinely require evaluation of the ability of a chemical to produce developmental neurotoxic effects in a pre-market setting.

Hormone-related cancers

- The increase in incidence of endocrine-related cancers in humans cannot be explained by genetic factors; environmental factors, including chemical exposures, are involved, but very few of these factors have been pinpointed.
- For breast, endometrial, ovarian and prostate cancers, the role of endogenous and therapeutic estrogens is well documented; this makes it biologically plausible that xenoestrogens might also contribute to risks. However, chemicals shown to be associated with breast (dioxins, PCBs and solvents) or prostate (unspecified agricultural pesticides, PCBs, cadmium and arsenic) cancer either do not have strong estrogenic potential or are unspecified. The possibilities of involvement of EDCs in ovarian and endometrial cancers have received little attention.
- For thyroid cancer, there are indications of weak associations with pesticides and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, but there is no evidence that hormonal mechanisms are involved.
- Models of hormonal cancers are not available for regulatory testing. This makes the identification

of hormonal carcinogens very difficult and forces researchers to rely on epidemiological studies. However, epidemiological studies cannot easily pinpoint specific chemicals and can identify carcinogenic risks only after the disease has occurred.

- Similar types of cancers of the endocrine organs, particularly reproductive organs, are also found in wildlife species (several species of marine mammals and invertebrates) and in domestic pets. In wildlife, endocrine tumours tend to be more common in animals living in polluted regions than in those inhabiting more pristine environments.

Adrenal disorders in humans and wildlife

- Experimental data and data from exposed wildlife populations suggest that both the hypothalamic–pituitary–adrenal (HPA) axis and the adrenal gland are targets for endocrine disruption caused by pollutants at environmentally relevant exposure concentrations; for example, adrenocortical hyperplasia is found in Baltic Sea seals exposed to a mixture of DDT and PCBs and their methyl sulfone metabolites. Despite this fact, and compared with other endocrine axes, the HPA axis has so far gained relatively little attention in endocrine disruptor research.
- Developing organs are particularly sensitive to alterations in hormone levels, and exposure to chemicals during critical windows of development may cause irreversible effects on the adrenal glands that may not be expressed until adulthood. Recent experimental data suggest that environmentally relevant exposures to pollutants (PCBs) affect development of the fetal adrenal cortex and the function of the HPA axis and induce delayed effects in the response to stress in animal models.
- For the great majority of chemicals, there is no evidence for effects of exposures on adrenal function, nor have there been any in vivo studies to test for this. A variety of chemicals and mixtures have, however, been shown to cause effects in vitro (in the H295R cell line).

Bone disorders

- Limited studies indicate that accidental poisoning of humans with hexachlorobenzene, PCBs and DDT caused bone disorders, and a plausible, although not proven, endocrine mechanism for these effects has been proposed.
- Epidemiological studies on humans also show a relationship between exposure to endocrine disrupting POPs and decreased bone mineral density or increased risk of bone fractures.

Metabolic disorders

- Obesity, diabetes and metabolic syndrome are due to disruption of the energy storage–energy balance endocrine system and thus are potentially sensitive to EDCs.
- Exposures of animal models to a variety of chemicals during early development have been shown to result in weight gain, revealing the possibility of an origin for obesity early in development. Because they are disrupting many components of the endocrine system involved in controlling weight gain (adipose tissue, brain, skeletal muscle, liver, pancreas and gastrointestinal tract), these chemicals constitute a new class of endocrine disruptors called “obesogens”.
- Obesity is also correlated with type 2 diabetes, and chemicals that have been shown to cause obesity in animal models also result in altered glucose tolerance and reduced insulin resistance.
- There are no compelling animal data linking chemical exposures with type 1 diabetes, although some chemicals can affect the function of insulin-producing beta cells in the pancreas, including BPA, PCBs, dioxins, arsenic and phthalates. Many of these chemicals are also immunotoxic in animal models, and so it is plausible that they could act via both immune and endocrine mechanisms to cause type 1 diabetes.
- Limited epidemiological data exist to support the notion that EDC exposure during pregnancy can affect weight gain in infants and children. Limited epidemiological data show that adult exposures to some EDCs (mainly POPs, arsenic, BPA) are associated with type 2 diabetes, but there are no data for type 1 diabetes, there is insufficient evidence of endocrine mechanisms and there is insufficient study of this area in general.

Immune function and diseases in humans and wildlife

- It is clear from both laboratory data and human and wildlife samples that EDCs can play a role in the development of immune-related disorders and are at least partially responsible for their rise in recent years.
- Since 2002, molecular mechanisms connecting a variety of nuclear receptors to NF-κB (one of the master regulators of inflammation and immunity) have been elucidated, and developmental immunotoxicity studies link compounds such as DES and the phytoestrogen genistein to postnatal immune disorders. Estrogen exposure has been shown to cause prostate inflammation, and BPA caused allergic sensitization, antibody production and type 2 helper T cell immune responses.

- Systemic inflammation, immune dysfunction and immune cancers such as lymphoma and leukaemia in humans have been associated with EDC exposures. These chemicals may exert their effects through nuclear receptor signalling pathways that have well-established ties with the immune system through cross-talk with inflammatory pathways.
- There are good epidemiological data associating exposure to polycyclic aromatic hydrocarbons, PCBs and other persistent POPs with autoimmune thyroid disease, exposure to phthalates and dioxins with endometriosis and allergies, and exposure to phthalates with asthma and other airway disorders. Endocrine mechanisms are not, however, clear.
- Together, these new insights stress a critical need to better understand how EDCs affect normal immune function and immune disorders and how windows of exposure may affect disease incidence (particularly for childhood respiratory diseases).

Population declines

- Wildlife species and populations continue to decline worldwide. This is due to a number of factors, including overexploitation, loss of habitat, climate change and chemical contamination.
- Given our understanding of EDCs and their effects on the reproductive system, it is extremely likely that declines in the numbers of some wildlife populations (raptors, seals and snails) have occurred because of the effects of chemicals (DDT, PCBs and tributyltin, respectively) on these species. The evidence for EDCs as a cause of these population declines has increased now relative to 2002, due to recoveries of these populations following restrictions on the use of these chemicals.
- EDCs in modern commerce with mechanisms of action similar to those of the endocrine disrupting POPs are suspected to also be a factor contributing to declines seen in wildlife species today. Demonstrating a clear link between endocrine effects in individuals and population declines or other effects will always be challenging, because of the difficulty in isolating effects of chemicals from the effects of other stressors and ecological factors. An endocrine mechanism for current wildlife declines is probable, but not proven.
- In spite of concerns about rising human populations on a global scale, numerous industrialized countries have fertility rates well below replacement levels. It has generally been accepted that socioeconomic factors play a role in these changes. It is plausible that widespread poor semen quality and subfertility levels also contribute to this trend; however, this has not been explored systematically.

Human and wildlife exposures to EDCs (chapter 3)

There is far more knowledge on exposure to EDCs and potential EDCs today compared with 10 years ago. This applies to the diversity of chemicals being implicated as EDCs and to the exposure routes and levels in humans and wildlife. As examples, brominated flame retardants were mentioned only briefly and perfluorinated compounds not at all when the IPCS document on EDCs was prepared 10 years ago (IPCS, 2002). In addition to these, there are now many more EDCs being found in both humans and wildlife. The most relevant main messages regarding exposure to EDCs follow:

- Unlike 10 years ago, it is now better understood that humans and wildlife are exposed to far more EDCs than just POPs. However, only a fraction of the potential EDCs in the environment are currently known.
- EDCs are chemically diverse, are primarily man-made chemicals and are used in a wide range of materials and goods. EDCs are present in food, nature (wildlife) and human beings. They can also be formed as breakdown products from other anthropogenic chemicals in the environment and in humans, wildlife and plants.
- Humans and wildlife are exposed to multiple EDCs at the same time, and there is justifiable concern that different EDCs can act together and result in an increased risk of adverse effects on human and wildlife health.
- Right now, only a narrow spectrum of chemicals and a few classes of EDCs are measured, making up the "tip of the iceberg". More comprehensive assessments of human and wildlife exposures to diverse mixtures of EDCs are needed. It should be a global priority to develop the capacities to measure any potential EDCs. Ideally, an "exposome", or a highly detailed map of environmental exposures that might occur throughout a lifetime, should be developed.
- Exposures to EDCs occur during vulnerable periods of human and wildlife development—from fertilization through fetal development and through nursing of young offspring—which raises particular concern.
- New sources of exposure to EDCs, in addition to food, have been identified and include indoor environments and electronics recycling and dumpsites (the latter being issues of particular concern for developing countries and countries with economics in transition). Children can have higher exposures due to their hand-to-mouth activities and higher metabolic rate.

- Not all sources of exposure to EDCs are known because of a lack of chemical constituent declarations for materials and goods.
- Spatial and temporal monitoring is critical for understanding trends and levels of exposure. This monitoring should include tissues from both humans and wildlife (representing a range of species) as well as water or other environmental compartments to capture the less persistent EDCs.
- Levels in humans and wildlife are related to how much a chemical is used. Bans on several POPs have led to declines in environmental levels and human body burdens. In contrast, there are increasing levels of some newer EDCs, such as perfluorinated alkyl compounds and replacements for banned brominated flame retardants.
- There is global transport of EDCs through natural processes (ocean and air currents) as well as through commerce, leading to worldwide exposure of humans and wildlife to EDCs.

Concluding remarks

EDCs have the capacity to interfere with tissue and organ development and function, and therefore they may alter susceptibility to different types of diseases throughout life. This is a global threat that needs to be resolved.

Progress

We are beginning to understand the importance of certain events during development and throughout the lifespan that interact with genetic background to increase susceptibility to a variety of diseases. It is clear that a large number of all non-communicable diseases have their origin during development. It is also clear that one of the important risk factors for disease is exposure to EDCs during development. Exposure to EDCs during development can, as demonstrated in animal models and in an increasing number of human studies, result in increased susceptibility to, and incidence of, a variety of diseases. These include some of the major human diseases that are increasing in incidence and prevalence around the world. The incidence of these diseases and dysfunctions is increased at current levels of exposure to EDCs in normal populations. It is also clear from human studies that we are exposed to perhaps hundreds of environmental chemicals at any one time. It is now virtually impossible to identify an unexposed population around the globe. There is an increasing burden of disease across the globe in which EDCs are likely playing an important role, and future generations may also be affected.

There have been clear benefits for human and wildlife health from the declining use of these chemicals. Government actions to reduce exposures, while limited, have proven to be effective in specific cases (e.g. bans and restrictions on lead, chlorpyrifos, tributyltin, PCBs and some other POPs). This has contributed to decreases in the frequency of disorders in humans and wildlife.

The advances in our understanding of EDCs have been based mainly on information derived from studies in developed regions. There is still a major lack of data from large parts of the world, in particular from Africa, Asia and Central and South America.

Future needs

Better information on how and when EDCs act is needed to reduce exposures during development and prevent disease from occurring. A clear example of the success of primary prevention through exposure control is lead. We have identified the following needs to take advantage of current knowledge to improve human and wildlife health by prevention of environmentally induced diseases.

A. Strengthening knowledge of EDCs: It is critical to move beyond the piecemeal, one chemical at a time, one disease at a time, one dose approach currently used by scientists studying animal models, humans or wildlife. Understanding the effects of the mixtures of chemicals to which humans and wildlife are exposed is increasingly important. Assessment of EDC action by scientists needs to take into account the characteristics of the endocrine system that are being disrupted (e.g. low-dose effects and non-monotonic dose–response curves, tissue specificity and windows of exposure across the lifespan). Interdisciplinary efforts that combine knowledge from wildlife, experimental animal and human studies are needed to provide a more holistic approach for identifying the chemicals that are responsible for the increased incidence of endocrine-related disease and dysfunction. The known EDCs may not be representative of the full range of relevant molecular structures and properties due to a far too narrow focus on halogenated chemicals for many exposure assessments and testing for endocrine disrupting effects. Thus, research is needed to identify other possible EDCs. Endocrine disruption is no longer limited to estrogenic, androgenic and thyroid pathways. Chemicals also interfere with metabolism, fat storage, bone development and the immune system, and this suggests that all endocrine systems can and will be affected by EDCs. Together, these new insights stress a critical need to acquire a better understanding of the endocrine system to determine how EDCs affect normal endocrine function, how windows of exposure may affect disease incidence (particularly for childhood respiratory diseases) and how these effects may be passed on to generations to come.

Furthermore, new approaches are needed to examine the effects of mixtures of endocrine disruptors on disease susceptibility and etiology, as examination of one

endocrine disruptor at a time is likely to underestimate the combined risk from simultaneous exposure to multiple endocrine disruptors. Assessment of human health effects due to EDCs needs to include the effects of exposure to chemical mixtures on a single disease as well as the effects of exposure to a single chemical on multiple diseases. Since human studies, while important, cannot show cause and effect, it is critical to develop cause and effect data in animals to support the studies on humans.

B. Improved testing for EDCs: Validated screening and testing systems have been developed by a number of governments, and it requires considerable time and effort to ensure that these systems function properly. These systems include both in vitro and in vivo end-points and various species, including fish, amphibians and mammals. New approaches are also being explored whereby large batteries of high-throughput in vitro tests are being investigated for their ability to predict toxicity, the results of which may be used in hazard identification and potentially risk assessment. These new approaches are important as one considers the number of chemicals for which there is no information, and these high-throughput assays may provide important, albeit incomplete, information. An additional challenge to moving forward is that EDC research over the past decade has revealed the complex interactions of some chemicals with endocrine systems, which may escape detection in current validated test systems. Finally, it will be important to develop weight-of-evidence approaches that allow effective consideration of research from all levels—from in vitro mechanistic data to human epidemiological data.

C. Reducing exposures and thereby vulnerability to disease: It is imperative that we know the nature of EDCs to which humans and wildlife are exposed, together with information about their concentrations in blood, placenta, amniotic fluid and other tissues, across lifespans, sexes, ethnicities (or species of wildlife) and regions. Many information gaps currently exist with regard to what is found in human and wildlife tissues, more so for developing countries and countries with economies in transition and for chemicals that are less bioaccumulative in the body. Long-term records to help us understand changes in exposures exist only for POPs and only for a few countries.

In addition, there is a need to continue expanding the list of chemicals currently examined to include those contained in materials and goods as well as chemical by-products; it is impossible to assess exposure without knowing the chemicals to target. The comprehensive measurement of all exposure events during a lifetime is needed, as opposed to biomonitoring at specific time points, and this requires longitudinal sampling, particularly during critical life stages, such as fetal development, early childhood and the reproductive years. Wildlife and humans are exposed to a wide variety of EDCs that differ greatly in their physical and chemical properties. Further, these compounds are generally present at trace concentrations and in complex matrices, requiring highly selective and sensitive analytical methods for their measurement. The wide range of different compound classes requires a variety of analytical approaches and techniques, making it challenging to understand all of the different chemicals in the environment and in human and wildlife tissues. There is a growing need to develop new analytical techniques and approaches to prioritize the assessment of EDCs. There is global transport of EDCs through natural processes (ocean and air currents) as well as commerce, leading to worldwide exposures. New sources of exposure to EDCs, in addition to food, have been identified and include indoor environments and electronics recycling and dumpsites (of particular concern in developing countries and countries with economies in transition). The sources and routes of exposure to EDCs need to be further investigated.

D. Identifying endocrine active chemicals: Identifying chemicals with endocrine disrupting potential among all of the chemicals used and released worldwide is a major challenge, and it is likely that we are currently assessing only the "tip of the iceberg". It is possible to trace high production volume chemicals, but that is not the case for the numerous additives and process chemicals. Adding greatly to the complexity, and to the number of chemicals in our environment, are the unknown or unintended by-products that are formed during chemical manufacturing, during combustion processes and via environmental transformations. While the active ingredients in pharmaceuticals and pesticides have to be documented on the final product, this is not the case for chemicals in articles, materials and goods. Personal hygiene products and cosmetics require declarations of the ingredients, and the number of chemicals applied in this sphere of uses counts in the thousands. Many sources of EDCs are not known because of a lack of chemical constituent declarations in products, materials and goods. We need to know where the exposures are coming from.

E. Creating enabling environments for scientific advances, innovation and disease prevention: Exposure to EDCs and their effects on human and wildlife health are a global problem that will require global solutions. More programmes are needed that foster collaboration and data sharing among scientists and between governmental agencies and countries. To protect human health from the combined effects of EDC exposures, poor nutrition and poor living conditions, there is a need to develop programmes and collaborations among developed and developing countries and those in economic

transition. There is also a need to stimulate new adaptive approaches that break down institutional and traditional scientific barriers and stimulate interdisciplinary and multidisciplinary team science.

F. Methods for evaluating evidence: There is currently no widely agreed system for evaluating the strength of evidence of associations between exposures to chemicals (including EDCs) and adverse health outcomes. A transparent methodology is also missing. The need for developing better approaches for evaluating the strength of evidence, together with improved methods of risk assessment, is widely recognized. Methods for synthesizing the science into evidence-based decisions have been developed and validated in clinical arenas. However, due to differences between environmental and clinical health sciences, the evidence base and decision context of these methods are not applicable to exposures to environmental contaminants, including EDCs. To meet this challenge, it will be necessary to exploit new methodological approaches. It is essential to evaluate associations between EDC exposures and health outcomes by further developing methods for which proof of concept is currently under development.

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Contents

Chapter 1

What is endocrine disruption all about?

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1.0 Introduction

We live in a world in which man-made chemicals are part of everyday life. Some of these chemical pollutants can affect the endocrine system, and as such can interfere with hormonally-controlled processes of humans and wildlife. In response to this recognition, the joint International Programme on Chemical Safety (IPCS) of WHO, UNEP and ILO (International Labour Organisation) developed in 2002 a "*Global Assessment of the State of the Science of Endocrine Disruptors*". In the intervening decade, a great deal of research has provided new information about the mechanisms by which environmental chemicals can interfere with hormone actions, the degree to which our environment is contaminated with such chemicals, and the relationship between chemical exposures and health outcomes in humans and in wildlife. The goal of this chapter is to provide an introduction to the concept of endocrine disruption before delving into the details of human and wildlife health effects in Chapter 2 and the exposure science in Chapter 3.

1.1 Overview of human and wildlife health

Chronic (non-infectious) diseases are the principal causes of sickness and death around the world (WHO, 2011; Hanson & Gluckman, 2011). In the pediatric population, this includes – but is not limited to – asthma, birth defects, neurodevelopmental disorders, cancer, diabetes and obesity (Bloom, Cohen & Freeman, 2009); in adults, this includes – but is not limited to – cardiovascular diseases (CVDs), cancer, diabetes and obesity, allergic and autoimmune diseases (Pleis, Ward & Lucas, 2010). Many of these diseases and disorders are increasing, some globally (WHO, 2011; Woodruff et al. 2004; reviewed in Chapter 2 of this document) (**Table 1.1**). Important examples are the increases in the global rates of obesity, elevated blood pressure, diabetes and metabolic syndrome. Taken together, chronic illness represents a significant burden on the world's populations (WHO, 2011).

As developed more fully in Chapter 2, the World Health Organization in 2008 estimated that 1.5 billion adults, aged 20 and older, were overweight and nearly 500 million were considered obese. In some developed countries like the USA, the prevalence reaches approximately 27% of adults and 17% of children and adolescents. Developing countries like Kuwait also have a very high prevalence and it is common to find obesity and malnutrition side by side in low- and middle-income countries. In the USA, the complications of obesity are now more financially costly than any other preventable cause of death with expenditures estimated to be 17% of all USA medical costs each year (Cawley & Meyerhoefer, 2012). Further, at age 12, obese children who remain overweight will have direct medical expenses throughout life associated with their excess weight that is estimated at US$ 6.24 billion (Trasande &Liu, 2011). Obesity is also a significant risk factor for other diseases and other disorders; worldwide estimates of billons of humans suffer from diseases associated with obesity such as glucose intolerance, insulin resistance, and raised blood pressure.

The number of diabetics in the world is expected to increase from 194 million in 2003 to 330 million in 2030 with three of four affected individuals living in developing countries. The global health expenditure on diabetes alone is expected to rise to US$ 490 billion in 2030–12% of all per capita health-care expenditures (Zhang et al., 2010). The burden of premature death from diabetes in developing countries is similar to that of HIV/AIDS, yet the problem is largely unrecognised in these areas.

Worldwide, an estimated 17 million people die of CVDs every year (mostly from heart attacks and strokes). Once associated with industrialized countries, CVDs are now emerging or rapidly increasing in some developing countries.

Alongside CVDs, adult cancers are also an increasing cause of mortality throughout the world and are exceeded only by CVDs in developed countries. As with CVDs also cancer frequency increases are strongly influenced by ageing. However, endocrine related cancers may not fully follow the same pattern. Breast cancer is the second most common cancer in the world and the most common among women. Other reproductive endocrine cancers such as prostate and cervical cancers are amongst the top ten most common cancers globally, together with colorectal, stomach, liver, oesophageal, head, neck and bladder cancers. The rates of breast, pancreatic, endometrial, prostate and kidney cancers are up to five times higher in industrialized countries than developing countries, whereas the rates of stomach cancer show decreasing trends with increasing economic development.

Limited data suggest difficulties among women to conceive and maintain pregnancy in the last two decades, (Swan et al., 1999; Chandra, 1998). Female reproductive disorders such as polycystic ovarian syndrome (PCOS), uterine fibroids and endometriosis are leading causes of sub fecundity and infertility, affecting 3 to 15%, 25-50%, and 10.35%, respectively, of women of reproductive age (Chapter 2.2). Large proportions (up to 40%) of young men in some countries have low semen quality which reduces their ability to father children (Chapter 2.3).

Table 1.1. Trends in childhood diseases and disorders. National statistics provide information on these trends.

Outcome	Years Available	Data Source	Data Description	Notes
Asthma	1980-2000	Centers for Disease Control and Prevention, National Center for Health Statistics, National Health Interview Survey	Data for ages 0-17. The NHIS is a continuing nationwide sample survey of the civilian non-institutionalized population collected by personal household interviews. In 2000, 32 374 people 18 years or older and 13 376 children aged 0-17 were interviewed. Data are based on parental response to whether child has had asthma in last 12 months (see text).	
ADHD	1997-2000	Centers for Disease Control and Prevention, National Center for Health Statistics, National Health Interview Survey	See NHIS description above. Data for ages 15-17. Terminology for this condition has evolved. The American Psychiatric Association adopted the name "attention deficit disorder" in early 1980s and revised it to "attention-deficit/hyperactivity disorder" in 1987. The NHIS of 1997-2000 used here to represent prevalence of ADHD used the term "attention deficit disorder". Data are based on parental response to the question, "Has a doctor or health professional ever told you that (child's name) had attention deficit disorder?"	Data for 1997-2000 are combined because of small response in single years. Data for children aged 5-17 are used because of difficulty in diagnosing ADHD in younger children
Mental retardation	1997-2000	Centers for Disease Control and Prevention, National Center for Health Statistics, National Health Interview Survey	See NHIS description above. Data for ages 0-17. Data are based on parental response to the to the question "Has a doctor or health professional ever told you that (child's name) had mental retardation?"	Most common definitions emphasize sub average intellectual functioning before 18 years of age, usually defined as IQ <70, and impairments in life skills. Different severity categories, ranging from mild retardation to severe retardation, are defined by IQ scores.
Childhood cancer	1974-1998	National Cancer Institute; Surveillance, Epidemiology and End Results Program (incidence); Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics system (mortality).		

There are also trends in pediatric health (Woodruff et al. 2004). In the United States, United Kingdom and Scandinavia, the preterm birth rate has increased by more than 30% since 1981. This is of concern because these infants experience increased rates of morbidity, including respiratory and neurological conditions, and mortality during the perinatal period. They are also more likely to suffer from CVDs and obesity, lung disease, and type 2 diabetes in adulthood (Chapter 2.2). In addition, birth defects are the leading cause of infant death and certain birth defects, such as those of the male reproductive organs are rising in many countries (Caione, 2009). Neurobehavioral disorders, including dyslexia, mental retardation, attention deficit hyperactivity disorder, and autism affect nearly 20% of children in those countries where it has been evaluated; autism spectrum disorders now occur at a rate that approaches 1% (Chapter 2.6). Thyroid diseases and disorders also represent a particularly high and increasing disease burden in children and adolescents in several countries in which they have been studied (Chapter 2.5). The prevalence of paediatric asthma has more than doubled over the past 20 years, and is now the leading cause of hospitalizations and school absenteeism (Landrigan & Goldman, 2011). The incidences of paediatric leukemia and brain cancer have also risen (Woodruff et al., 2004), as well as the incidence of testicular cancer (increases of up to 400%), the most common cancer in young men in many industrialized countries (Chapter 2.3). Aside from these disease trends, there is a secular trend toward premature puberty among American and European girls which is concerning because it can lead to reduced adult height, increased risk of breast cancer and polycystic ovarian syndrome, and a greater likelihood of engaging in risky behaviours (i.e. smoking, unprotected sex, alcohol and drugs; Chapter 2.2).

These public health statistics have important parallels in some wildlife populations. For example, testicular non descent was observed in 68% of males in a population of black deer in Alaska; similar trends were also observed in Montana. There is recent evidence that animals living near humans have trended toward increasing body weight (Klimentidis et al., 2011). All of these diseases have both a genetic and an environmental component and because the increase in incidence and prevalence cannot be due solely to genetics, it is important to focus on understanding the contributions made by the environment, often easier to study in wildlife than in

human populations. For example, as early as 1915, lead-related neurological disorders were observed in horses and cattle living near industrial facilities; adverse neurological effects of mercury were seen in the local wildlife species in Minimata Bay (Japan) before they were seen in the human residents there and in those living in the Great Lakes basin (Chapter 2.6). Differences in mammary cancer prevalence between carnivores and herbivores and between captive and wild carnivores are striking and support the hypothesis that diet is a major risk factor for these cancers. In the St Lawrence estuary, both the beluga whale and human populations were affected by higher rates of cancer than populations in other parts of Quebec and Canada, and some of these cancers were epidemiologically related to exposure to chemical contaminants, also observed in the beluga whale. In another example, a study of more than 8 000 dogs showed that canine bladder cancer mimicked the distribution of bladder cancer among their human owners (Chapter 2.7.4).

More recent evidence of environmentally caused human diseases and disorders may exist in wildlife species. For example, an inverse association between mercury exposure and DNA methylation in the brain stem of Greenland polar bears may be related to a similar association between mercury exposure and neurological deficits in Inuit children (Chapter 2.6). Genital malformations, lowered semen quality and altered sex hormone levels seen in male fish in urban areas and amphibians in agricultural areas appear to mirror those observed in human populations in similar environments and may reflect common causes (Chapter 2.3). The apparent similarities between diseases and disorders reported in humans and in various wildlife populations are not surprising given that there is often considerable overlap between their environments and food chains as well as in their physiology.

Prüss-Üstün and Corvalán have estimated that as much as 24% of human diseases and disorders globally are due at least in part to environmental factors (Prüss-Üstün & Corvalán, 2006). This provides both a challenge to identify and address, but also a tremendous opportunity to improve human and wildlife health by improving elements of the environment that impact public and wildlife health (Landrigan & Goldman, 2011). The recognition of these challenges and opportunities, along with the fact that many of the most prevalent diseases are associated with the endocrine system, has led to a focus on chemical exposures and specifically endocrine disruptors; a subclass of chemicals that act by disrupting the normal functioning of the endocrine system.

Attention has focused increasingly over the past 20 years on the hypothesis that environmental chemicals may cause human and wildlife diseases by interfering with normal hormone action. In 2002, UNEP, in collaboration with WHO, brought together a group of scientists knowledgeable about research on endocrine disruptors to produce the IPCS Global Assessment of the State of the Science of Endocrine Disruptors document (IPCS, 2002). Since that time, a great deal of scientific work has improved our understanding of each of these issues, and these will be highlighted below. Research published over the past 10 years has confirmed the scientific complexity of endocrine disruption. As an index of the scientific complexity of this issue, the Endocrine Society published a scientific review in 2009 that cited nearly 500 scientific articles focused on various aspects of endocrine disruption (Diamanti-Kandarakis et al., 2009).

The goal of the current document is to update the 2002 IPCS document, providing a current state-of-the science of endocrine disruptors as it relates to human and wildlife population health. It is essential to frame the issue of EDCs within the context of normal endocrine function; therefore in Chapter 1, we begin with a discussion of hormones and human and wildlife health. In Chapter 2, we provide a detailed scientific review of the literature concerning human and wildlife endocrine disorders and diseases plausibly impacted by endocrine disruptors. And in Chapter 3, we review the literature documenting the exposure of humans and wildlife to environmental chemicals with endocrine disrupting properties. The following review of the state of the science of endocrine disruption was developed by a large number of experts who have contributed significantly to the primary literature, and who have an international reputation for their work.

1.2 What are hormones?

To understand endocrine disruption, we must understand the basic features of the endocrine system; a series of ductless glands that secrete hormones directly into the blood to regulate various body functions. The traditional definition of a hormone is a molecule produced by an endocrine gland that travels through the blood to produce effects on distant cells and tissues (Melmed & Williams, 2011). Traditional concepts of endocrine glands include the pituitary gland at the base of the brain, the thyroid gland in the neck, the adrenal glands in the abdomen next to the kidneys, the gonads and certain parts of the pancreas. In addition to these specialized endocrine glands, many other organs that are part of other body systems, such as the heart, body fat, muscle, liver, intestines, and kidneys – have secondary endocrine functions and also secrete hormones (**Figure 1.1**).

Hormone effects are mediated by specific proteins called receptors (defined in section 1.2.2). Without receptors, hormones cannot exert their hormonal effects. Steroid hormones tend to be carried through the blood by specific “carrier” proteins, and are able to passively enter cells and interact with receptors inside the cells. In contrast, protein and amine hormones cannot passively enter cells, so specific mechanisms must be in place to allow these hormones to affect their target organs and cells and this usually involves interactions with cell membrane associated receptors on the outside of the cell. Thyroid hormones are unique in that they act on receptors inside cells, but require specific transport proteins to gain access to the inside of a cell, unlike steroid hormones. We are learning that steroid hormones can also act through cell membrane receptors and this is likely to be important to fully understand their effects and to understand the ways in which exogenous chemicals can interfere with their actions.

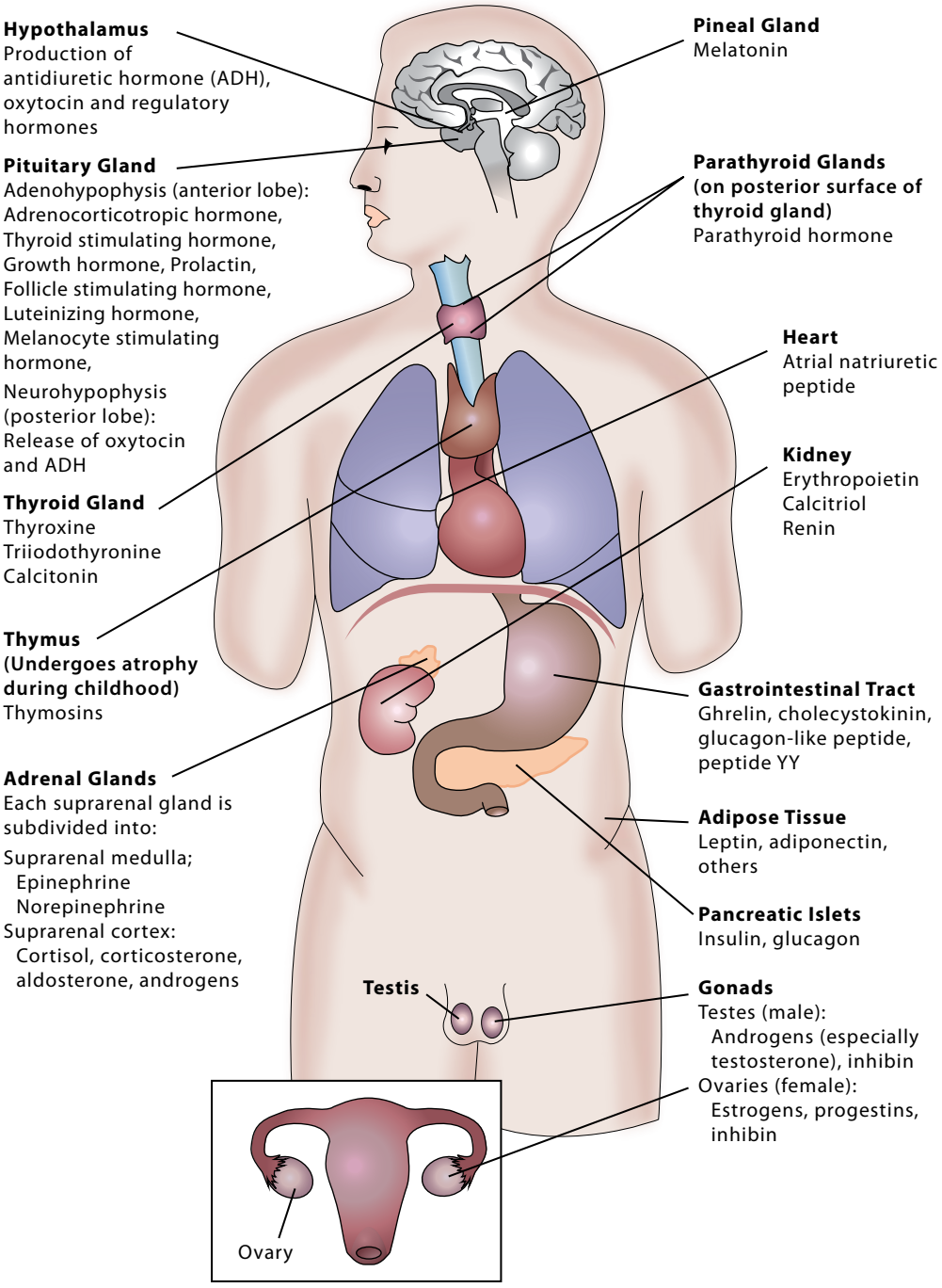

Figure 1.1. What are hormones? Hormones are molecules produced by specialized cells in a large variety of glands and tissues. These molecules travel through the blood to produce effects at sometimes distant target tissues.

1.2.1 Hormones control major physiological processes

Hormones are important to both vertebrates and invertebrates. They are essential for controlling a large number of processes in the body from early processes such as cell differentiation during embryonic development and organ formation, to the control of tissue and organ function in adulthood (Melmed & Williams, 2011). A well-known example is that of insulin, a small protein hormone produced by specialized cells in the pancreas called “beta cells”. These cells are stimulated to secrete insulin into the blood by the direct action of the sugar, glucose. As blood levels of glucose rise during and after a meal, it enters the beta cells through a specific protein transporter on the cell membrane and is converted inside the cell to the high-energy compound ATP. This process directly causes changes inside the beta cells, resulting in the secretion of insulin that was already produced and stored in anticipation of these events. Insulin then travels through the blood to many different tissues and cells, causing glucose to be taken up into those tissues via specific membrane receptors linked to transport systems.

As a result, blood glucose levels fall, which then shuts off insulin secretion from beta cells. In this way, insulin is important not simply to maintain glucose levels within a fairly narrow range in the blood, but it is important for tissues to be able to take up and use glucose for energy. In addition, insulin reaches the brain, where it has important effects on appetite. Because insulin rises during and after a meal, this hormone plays a role in regulating the feeling of hunger. This is typical of hormones –

Table 1.2. Basic overview of the endocrine system and hormones (not comprehensive).

Hormone System	Hormone	Actions
Ovary	Progesterone Estrogens (converted from androstenedione)	Timing of ovulation
		Supports pregnancy
		Ovulation, secondary sex characteristics, uterine growth
Testes	Androgens	Maturation of sex organs, secondary sex characteristics, body size
Placenta & uterus (during pregnancy)	Progesterone	Supports pregnancy
	Chorionic gonadotropin	Promotes maintenance of corpus luteum.
	Prolactin	Coordinates thyroid function
		Promotes growth of mammary gland & milk production
Thyroid	Thyroxine (T4)	Major product of thyroid gland, metabolism development
	Triiodothyronine (T3)	Hormonally active form of T4
	Calcitonin	Regulates blood calcium levels, stimulates bone construction
Parathyroid	Parathyroid hormone (PTH)	Regulates blood calcium levels
Pituitary gland	Growth hormone (GH)	Stimulates growth
	Thyroid stimulating hormone (TSH)	Stimulates T4 production by thyroid gland
	Follicle stimulating hormone (FSH)	Stimulates follicle maturation in ovary, stimulates spermatogenesis in testes
	Luteinizing hormone (LH)	Stimulates ovulation (females), testosterone synthesis (males)
Hypothalamus	Thyrotropin releasing hormone (TRH)	Promotes secretion of TSH and prolactin by pituitary
	Growth hormone releasing hormone	Stimulates secretion of GH from pituitary
	Growth hormone inhibiting hormone (Somatostatin)	Inhibits release of GH from pituitary
	Gonadotropin releasing hormone (GnRH)	Stimulates secretion of FSH and LH from pituitary
	Corticotropin Releasing Hormone (CRH)	Stimulates ACTH secretion from the pituitary gland
	Vasopressin	Has pressor effect on the cardiovascular system and is a major anti-diuretic hormone
	Oxytocin	
	Dopamine	Causes smooth muscle contraction including the uterus during parturition and in milk let-down
		Inhibits Prolactin secretion
Stomach	Gastrin	Causes secretion of gastric acid
	Ghrelin	Stimulates appetite
Pancreas	Insulin	Uptake of glucose, regulates glycolysis
	Glucagon	Release of glucose, regulates gluconeogenesis
	Somatostatin	Inhibits release of insulin and glucagon
Liver	Insulin-like growth factor (IGF)	Regulates cell growth, has insulin-like properties
Adipose tissue	Leptin	Decreases appetite, increases metabolism
Kidney	Renin	Regulates blood pressure & fluid balance
	Erythropoietin	Stimulates production of red blood cells
Adrenal gland	Cortisol	Stimulates gluconeogenesis, fat metabolism, inhibits glucose uptake into cells
	Aldosterone	Stimulates water resorption, controls blood pressure & fluid balance
	Adrenaline/epinephrine	Boosts oxygen and glucose to brain & muscles, suppresses non-emergency body responses
	Noradrenaline/Norepinephrine	Boosts oxygen and glucose to brain & muscles
	Dopamine	Regulates heart rate & blood pressure

they are primarily involved in important physiological processes, but they also act on the brain to integrate various behaviours with the specific physiological processes.

In the same way, other hormones control major physiological functions and coordinate these functions between systems (**Table 1.2**). Reproductive hormones, steroids (estrogens, androgens, progestins) and proteins (LH and FSH) control the complex physiological processes associated with reproduction. Thyroid hormones control metabolic processes and coordinate these with the many hormones involved in appetite and body weight regulation and metabolism. The adrenal hormones control the various physiological responses to stress. In addition to their actions on these physiological processes, many hormones also control their own secretion by "negative feedback". For example, thyroid hormone secretion is stimulated by a pituitary protein hormone, TSH (thyroid stimulating hormone), and thyroid hormones in turn suppress TSH. In this way, thyroid hormone levels are maintained within a relatively narrow range for an individual under normal circumstances. All hormone systems are governed to some extent by these processes so that hormone levels are at the appropriate concentration in blood to be effective at controlling physiological process. However, it is also important to note that there are times when tissues can control hormone action locally such that the level of hormone in the blood is not indicative of hormone action in the tissues (see below).

A wide variety of developmental problems and common adult diseases and disorders are well-known to be caused by abnormal endocrine function. For example, diabetes is the result of a defect or defects in insulin action. Defects might be caused by insulin not being present, being present in insufficient or excess amounts, or by a defect in the receptor that mediates insulin action. The insulin itself may be mutated, or there may be a mutation in the receptor or other proteins that are essential for insulin to act properly. Like most non-communicable diseases, diabetes is a result of a complex combination of genetic processes and the environment. Therefore, we need to measure and assess both types of factors to better understand complex disease. The wealth of knowledge that has been gained by studying hormone systems and endocrine-related diseases has enhanced our ability to treat people with these diseases and disorders and forms the basis of our ability to identify environmental chemicals that can interfere with hormone action and evaluate the consequences of exposure to these chemicals.

1.2.2 Hormones act on receptors

Hormones produce effects by acting on specialized proteins called receptors (see **Figure 1.2**), which attract and bind to specific hormones. Hormone receptors provide specificity to hormone actions, both in terms of the time and the place of hormone action. Hormone receptors are always limited in their abundance and cause limited and specific effects downstream of hormone binding. Most hormones do not act in all cells because their receptors are not found in all cells. Most hormones also do not act at all times during the life cycle because their receptors are found only at specific times during development or in adulthood.

Importantly, hormones do not act the same way in all cells or in the same cell at different developmental times, and we are beginning to understand the physiology behind these differences. In some cases, multiple receptor types mediate the actions of a single hormone. For example, there are two receptor classes for thyroid hormones (TRα and TRβ), and they have different temporal and spatial patterns of expression. The spatial distribution of hormone receptors can vary widely and this accounts for the degree to which the hormone has global effects. For example, insulin receptors are found throughout the body and this accounts for the ability of insulin to affect all tissues. In contrast, receptors for the hormone Thyroid Stimulating Hormone (TSH) are found predominantly in the thyroid gland. This limited distribution accounts for the much more restricted impact of TSH in the body.

Likewise, estrogens exert their effects by acting on at least two major nuclear receptor types (Estrogen Receptor alpha and beta; ERα and ERβ), although they also act by specific membrane receptors on some cells. There is still uncertainty about how many different kinds of receptors mediate estrogen actions. In contrast, testosterone exerts its effects by acting on a single Androgen Receptor (AR). Despite the fact that some hormones act through multiple receptors and some hormones act through a single receptor, in all cases the actions of a hormone in one cell type are different from the actions of that hormone in another cell type. There are important processes that contribute to the cell-specific nature of hormone actions, and it is important to understand how this happens so that the effects of environmental chemicals that interfere with these processescan be better predicted.

In the case of nuclear receptors – receptors for steroid and thyroid hormones – the hormone-receptor complex binds to specific regions of DNA to regulate the process of gene transcription resulting in the formation of new proteins (**Figure 1.2**). These hormones regulate different genes in different cell types, or at different times during development. While this flexibility is in part due to having different receptor types in different cells, there are also mechanisms that allow the same receptor to have different effects in different cells. These mechanisms are not completely understood, but they include mechanisms that turn "off" or "on" specific genes independent of the hormone. Thus, a liver cell will produce different proteins than a brain cell, despite the fact that the same hormone (e.g. estrogen) can affect both cells.

Protein hormone receptors can also be located on the cell membrane (**Figure 1.2**). Insulin, for example, binds to its membrane receptor to cause cells to take up and use glucose. After insulin binds to its receptor, there are very specific responses inside the cell to cause glucose to be taken up. In this regard, it is important to recognize that insulin causes glucose uptake by different processes in different cells, even though there is only one insulin receptor. For example, insulin stimulates the production of glycogen in the liver (which sequesters glucose inside liver cells), but it activates a glucose

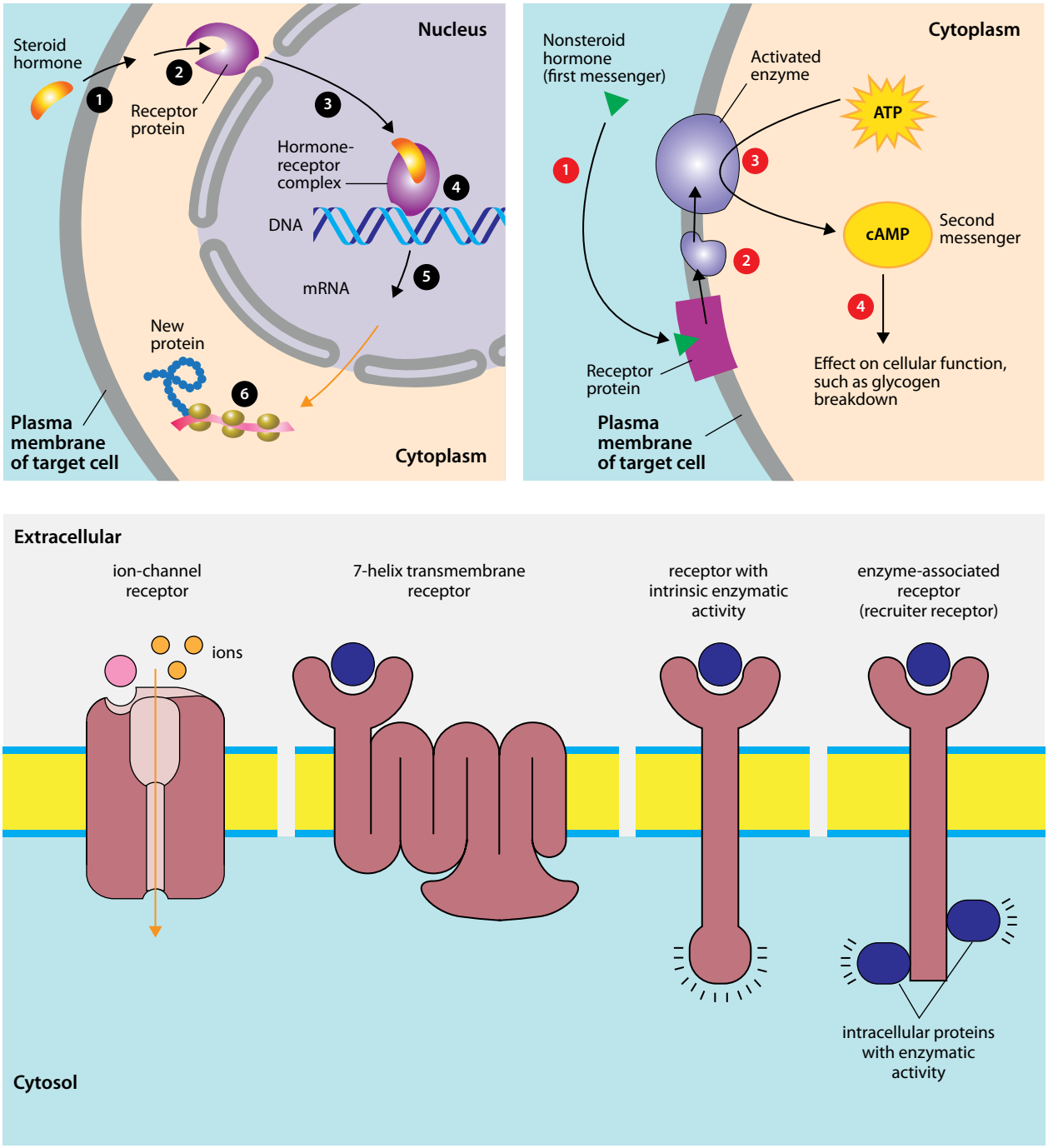

Figure 1.2. Hormones produce effects in the body exclusively by acting on receptors. There are different classes of receptors. A (Upper left): Nuclear receptors bind to steroid and thyroid hormones and act directly to regulate gene expression. B (Upper right): Membrane receptors bind to protein and amine hormones and produce effects inside the cell by a second messenger system. C (lower): Membrane receptors can be linked to a variety of second messenger systems. In addition, there are "co-regulator" proteins that link the hormone receptor to the transcriptional apparatus, and these co-regulators can differ between cells, which can affect the way a nuclear hormone receptor can function. These are important considerations because we know that environmental chemicals can interact directly with some nuclear receptors in ways that change their ability to interact with gene regulatory processes, thereby producing effects that are unexpected. These effects need to be identified and considered when we think about endocrine disruption.

transporter in other tissues, which directly stimulates glucose uptake. This occurs because the insulin receptor is linked to different kinds of cellular machinery in different cells. All protein and peptide hormones act in a similar fashion; there are specialized receptors on the outside of cells that "transduce" the effect of hormone binding to the inside of the cell. These receptors are linked to different sets of proteins in different cells, which cause the cells to respond differently to the same hormone. Interestingly, there are also membrane receptors for some steroid hormones that also act via nuclear receptors. Estrogens and progestins both act through nuclear and membrane receptors. In these cases the membrane receptor is

coupled to fast acting pathways that result in immediate effects, in contrast to the nuclear receptors, which take several hours to stimulate production of new proteins and exert effects.

Importantly, hormone receptors on the cell membrane can interact with hormone receptors in the nucleus by various mechanisms. Thus, different kinds of hormones can interact with each other through both extracellular and intracellular receptors to regulate development and various physiological processes.

1.2.3 Hormones act at very low concentrations

In general, hormones act at very low concentrations. In part, because hormones act through high affinity receptors; that is, very low concentrations of hormone can bind to the receptor population and initiate important biological effects. In addition, hormones produce a sigmoidal dose-response curve (see **Figure 1.3A**). In this case, small changes in hormone concentration at the low end of the dose-response curve produce greater differences in effect than similar changes in hormone concentration at the high end of the dose-response curve. This is important because very low concentrations of environmental endocrine disruptors could add to the endogenous hormone effect to produce a response that is much greater than would be predicted based on the hormone alone. In addition, hormone receptors can be expressed in a single cell at different concentrations, and this will affect the various characteristics of the dose-response curve (see **Figure 1.3B**). In this case, as receptor concentration increases, the dose-response curve is shifted to the left; i.e., the same biological effect is produced at lower hormone concentrations. This can explain both why some endpoints of hormone action are more sensitive to hormones than others, and can explain why some are more sensitive to exogenous chemicals than others.

1.2.4 Responses to hormones are not linear and can be "bi-phasic" (non-monotonic)

Because hormones interact with and activate their receptors in a non-linear fashion, dose-responses are at least sigmoidal, but can also be more complex, including being non-monotonic (**Figure 1.3C**). These dose response curves are often referred to as U-shaped (with maximal responses observed at low and high doses) or inverted U-shaped (with maximal responses observed at intermediate doses). In vitro studies have been instrumental in understanding the mechanisms behind non-monotonic dose responses. Studies using many hormone sensitive cell lines have shown that non-monotonic responses can be produced by a variety of mechanisms. One mechanism involves integrating two (or more) monotonic responses that affect a common endpoint. For example, studies of prostate cell lines have shown that these cells proliferate to the highest degree when provided with intermediate concentrations of androgen. The reason for this inverted U-shaped response is

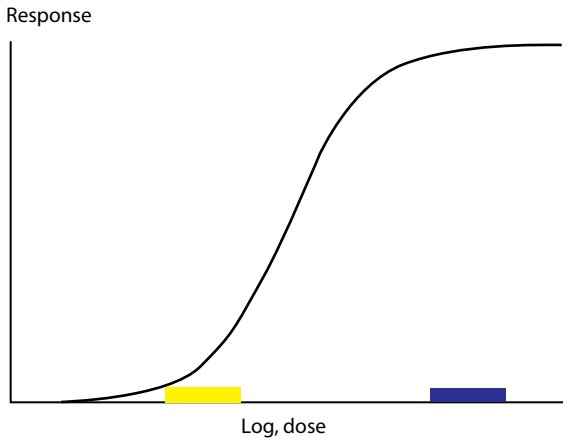

Figure 1.3A. Dose-response curve for hormones. As the dose of hormone increases, the response increases in a logarithmic manner until the point of saturation. Different hormone-receptor interactions will have differences in the dose of hormone or the dynamic range of the log-linear portion of the curve or the maximal response. Some receptors are down-regulated by the hormone, so the dose-response curve will decline at the high dose (this will be a function of both dose and time). Note that a small change in hormone concentration at the low end of the curve (yellow box) will have much greater effects on the response than a similar change in hormone concentration at the high end of the curve (blue box).

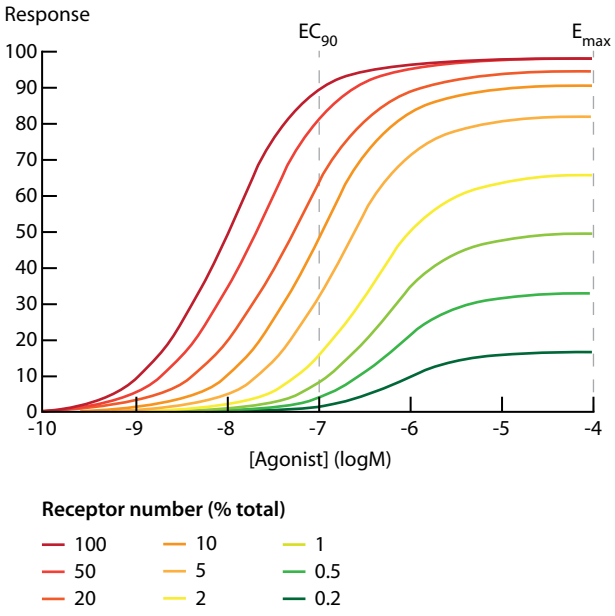

Figure 1.3B. The dose-response to the hormone depends on receptor concentration. These data show clearly that as the receptor concentration increases, the hormone becomes "more potent"; that is, it takes significantly less hormone to produce the same response. In fact, at low hormone receptor levels, the maximum response does not achieve the "EC50" response of the high receptor level. (Figure from Charlton (2009), redrawn; Used with publisher's permission).

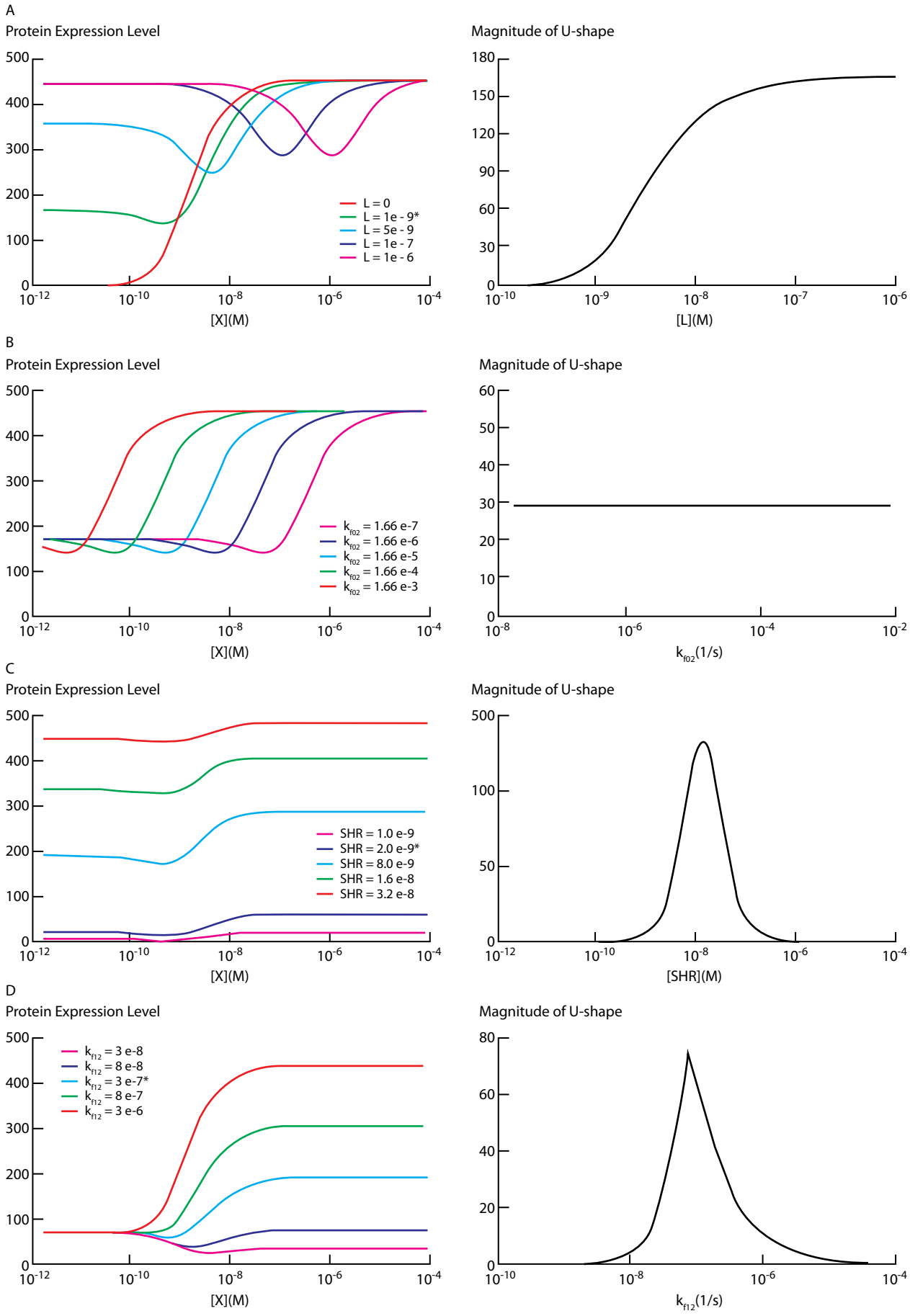

Figure 1.3C. Hormones can produce dose-response curves of various shapes including non monotonic. Non-monotonic dose-response relationship in steroid hormone receptor-mediated gene expression. (Figure from Li et al. (2007), redrawn; Used with publisher's permission).

that the cell line actually contains two populations of cells: one population proliferates in response to testosterone, while testosterone inhibits cell proliferation in the other population. At low doses, the first population has minimal proliferation, and at high doses the second population has a low level of proliferation because it is being inhibited. When looking only at cell number, intermediate doses have the maximal effect because at these concentrations the first population is somewhat proliferative and the second population is only somewhat inhibited. Ultimately, these two cell populations were isolated from each other, and when observed individually, each one had a monotonic response to androgen. Vandenberg et al. (2012) have extensively reviewed these issues.

Non-monotonic dose responses also occur as the result of receptor down regulation. When hormones are present in high concentrations, they bind to their receptors causing a down-regulation of receptor number. The degradation of receptors is increased when the hormone is abundant, and the cell's ability to replace these receptors is slower than the rate at which they are removed from the system. Thus, high concentrations of hormone lead to fewer available receptors, and a natural shift in the receptor-mediated response. In addition to this mechanism, non-monotonic responses can be caused by the increased toxicity of a hormone (cytotoxicity) at high doses. For example, the MCF7 breast cancer cell line proliferates in response to estrogen until high doses are reached (10^{-5} – 10^{-4} M) where it is cytotoxic resulting in cell death. The same toxicity has been observed in a subpopulation of MCF7 cells that no longer express the estrogen receptor, suggesting that 17β-estradiol (the natural estrogen) is not having endocrine effects at these high doses, but is generally toxic.

Finally, non-monotonic dose responses can occur because of differences in receptor affinity at low versus high doses. More specifically, low doses of estrogen bind almost exclusively to estrogen receptors, but at high doses it can also bind weakly to other hormone receptors, like androgen receptor and thyroid hormone receptor. Hormone-receptor complexes are often thought of as a lock-and-key, suggesting that the 'keys' (hormones) can actually fit multiple 'locks' (receptors) at certain concentrations. Thus, the effects seen at high doses can be due to action via the binding of multiple receptors, compared to the effects of low doses, which are only caused by action via a single receptor or receptor family.

1.2.5 When do hormones act?

Hormones act at all times during life – in utero and in early life, in childhood, puberty, adulthood and in aging (**Figure 1.4**). In fact, the timing of hormone action is an important determinant in the potency of hormone action at low doses. In the adult, hormones are thought to have transient effects on target cells and tissues. Thus, the hormone has an effect when it is present, but when the hormone is withdrawn the effect diminishes – much like insulin levels rising when blood sugar is high, and then declining when blood sugar declines. This does not diminish their importance, but contrasts with their effects in the fetus and neonate where a hormone can have permanent effects in triggering early developmental events such as cell proliferation or differentiation. Hormones acting during embryonic development can, cause some structures to develop (e.g. male reproductive tract) or cause others to diminish (e.g. some sex-related brain regions). Once

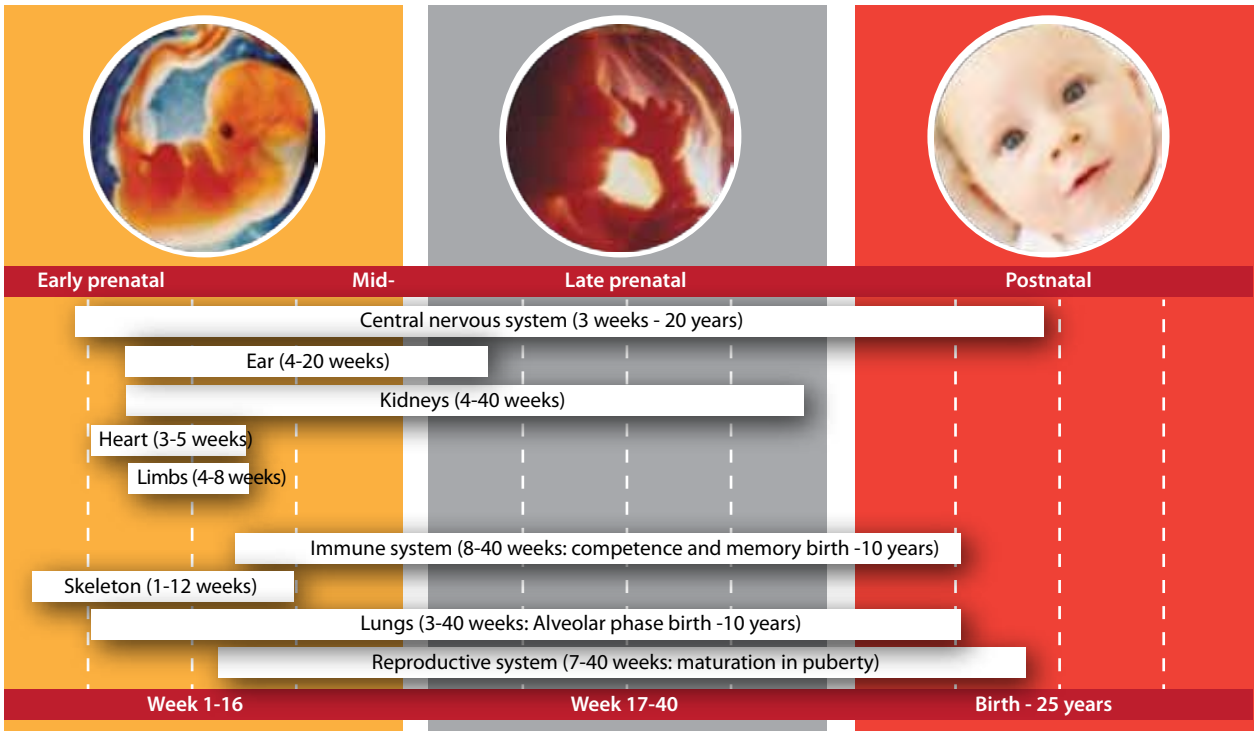

Figure 1.4. Timing of organ development. Hormones affect each of these indicating that they are important, and in different ways, throughout life.

hormone action has taken place, at these critical times during development, the changes produced will last a lifetime.

In some ways, hormone actions during development are considered to be programming events. The term "programming" in this case refers to the ability of hormones to exert effects in the fetal period that influence or dictate the functions of endocrine and physiological systems in the adult. For example, the monthly cycle of changes in hormone levels in adult women that cause ovulation, and upon which female fertility depends, is programmed during fetal development by the actions of estrogens and androgens (reviewed in Mahoney & Padmanabhan, 2010; Sarma et al., 2005)). Thus, small perturbations in estrogen action during fetal development can change the reproductive axis in adulthood and diminish fertility (Mahoney & Padmanabhan, 2010). It is now clear that fetal programming events can predispose the adult to a number of chronic diseases (Janesick & Blumberg, 2011; Hanson & Gluckman, 2011); thus, endocrine disease prevention should begin with maternal and fetal health. In some cases, experimental studies have identified the developmental events that influence adult function. An important example of this is that of thyroid hormone and brain development. It is well established that thyroid hormone is essential for brain development during the fetal and neonatal period in humans, especially as revealed in the disorder known as congenital hypothyroidism (CH) (Klein, 1980). In fact, all babies born in developed countries are universally screened for thyroid function to identify those children with a defective thyroid gland (Klein, 1980). This strategy has been successful in preventing severe mental retardation. Moreover, the fundamental knowledge we now have of the mechanisms by which thyroid hormone acts and the developmental events it controls can further guide clinical management of these and other thyroid disorders.

1.3 What are endocrine disruptors?

In the preface to the 2002 IPCS document, endocrine disruptors were referred to as "*…chemicals that have the potential to interfere with the endocrine system*"; in Chapter 1 of that document, an endocrine disruptor was defined in a generic sense as, "*…an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. A potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations.*" (IPCS, 2002). While there have been many modifications of this definition (Kortenkamp et al., 2011), we use the original for this review. However, scientific reports over the past decade support the conclusion from the ICPS 2002 document that endocrine disruptors represents a unique kind of toxicity.

If an endocrine disruptor is an exogenous chemical that alters the function(s) of the endocrine system and thereby causes adverse health effects, it is important to consider the role of hormone action in development and adult physiology as the backdrop for understanding both the potential health consequences in a population (human or wildlife) and the ways that we can observe this activity in experimental systems and in human epidemiology. The mechanism by which a chemical disrupts hormone action has a very large impact on the pattern of effects one would expect to observe. Generally, there are two pathways by which a chemical could disrupt hormone action: a direct action on a hormone receptor protein complex, or a direct action on a specific protein that controls some aspect of controlling hormone delivery to the right place at the right time. This could be a protein that is involved in hormone production (e.g. aromatase), an important transporter (e.g. sodium/iodide symporter), or a carrier protein (e.g. cortisol binding protein). Thus, a chemical could block the synthesis of a hormone, with the result that the blood levels of the hormone would increase or decline. The impact on the downstream action of that hormone would likely be the same as the situation in which hormone levels are changed because of disease or genetic defect in which hormone synthesis is inhibited or stimulated. In contrast, if a chemical interacts directly with a hormone receptor, then the effects could be quite complex and should be expected to follow the mechanisms outlined above for how hormones interact with receptors.

In the following sections, we introduce the evidence that exogenous chemicals can interfere with hormone action and produce adverse effects. It is not our intention to develop a list of known EDCs, or to identify the properties of EDCs. Rather, we provide a description of the logic that must be implemented to identify EDCs and their properties.

1.3.1 Endocrine disruptors act on major physiological systems

Endocrine disruptors interfere in some way with hormone action, and in doing so can produce adverse effects on human and wildlife health. The physiological systems affected by this disruption likely include all hormonal systems ranging from the development and function of reproductive organs to adult onset diabetes or cardiovascular disease. While there are many hormones and hormone systems (see **Figure 1.1**), most studies of endocrine disruptors have focused predominantly on chemicals that interact with estrogen, androgen and thyroid hormone systems. A growing number of studies, however, indicate that environmental chemicals can interfere with other endocrine systems (Casals-Casas & Desvergne, 2011); indeed, no endocrine system should be ruled out for being directly affected by environmental chemicals. Fat development and weight gain is a good example of complex physiological systems that are influenced by endocrine disruptors. There are a number of endocrine disruptors that have been shown to affect weight gain, insulin sensitivity and glucose tolerance (See Chapter 2 for details) indicating a potentially important role for endocrine disruptors in the development of obesity,

Table 1.3. Comparison of hormone and endocrine disruptor action.

Hormones	Endocrine Disruptors
Act via receptors	Some act on hormone receptors
Some have multiple receptors	Will cause abnormal receptors function
Tissue specific receptor classes and subtypes	Likely isoform-specific interactions
Hormones normally bind similarly to all receptor subtypes	
Active at low doses	Some act at low doses, others variable
Blood levels do not always reflect activity	Blood levels do not always reflect activity
May be bound to serum proteins in blood with small % free	May be bound to serum proteins
	Effects on hormone blood levels may not reflect on hormone action
No bioaccumulation	Possible bioaccumulation
Non-linear dose response relationships	Non-linear dose response relationships
Always saturable with variable dynamic range	Always saturable with variable dynamic range
Can exhibit non-monotonic dose-response	Can exhibit non-monotonic dose-response
High dose effects not same as low dose	High dose effects not same as low dose
Tissue and life-stage specific effects	Tissue and life-stage specific effects
Developmental effects permanent	Developmental effects permanent
Programs brain and endocrine system for adult function	Interferes with programming processes
Different end-points vary in sensitivity	Different end-points vary in sensitivity

type 2 diabetes and metabolic syndrome (Casals-Casas & Desvergne, 2011). The elements of the endocrine system that control weight gain and metabolism/energy expenditure include the adipose tissue, pancreas, GI tract, liver, skeletal muscle, bone and brain, and endocrine disruptors could specifically and directly affect each of these tissues by interfering with their various hormone systems.

1.3.2 How do exogenous chemicals interfere with hormone action?

As discussed above, environmental chemicals have been shown to exert direct actions on hormone receptors and receptor function, as well as to exert direct actions controlling hormone delivery to the receptor. Both of these pathways of endocrine disruption can be quite specific to a particular endocrine system in part because hormone receptors have features that might be somewhat unique to that system, and because the process by which a hormone is delivered to the target may be somewhat unique. Thus, there are few rules about the actions of endocrine disruptors that can be generalized to all cells and organs of the body and to all times in the life cycle. Since endocrine disruption represents a special form of toxicity, this must be taken into consideration when interpreting the results of studies of endocrine disrupting chemicals, or when designing studies to clarify the effects of endocrine disrupting chemicals and quantifying the risks to human and wildlife health.

Hormone receptors have a high affinity for their natural ligand, but typically a much lower affinity for endocrine disruptors – with some exceptions. However, it is important not to confuse *affinity* (ability to bind) for the receptor with *potency* (ability to cause effects) of action (Ruenitz et al., 1996). Although we don't fully understand this issue, the part of the chemical that controls its ability to bind to the receptor is not necessarily the same part of the chemical that controls receptor activation. Also, as reviewed in section 1.2.3, receptor abundance will impact the dose at which a hormone activates the receptor, and this will apply to an endocrine disruptor as well. This may underlie in part the tissue- or cell-specific differences in effects of endocrine disruptors. Thus, just like the hormones they interfere with, endocrine disruptors will be receptor and tissue specific. Some endocrine disruptors actually have an affinity that is similar to or greater than that of the natural ligands. An example of this is tributyltin (TBT), which has an affinity for RXR (retinoid-X-receptor) and PPARγ (peroxisome proliferator activating receptor subtype gamma) in the low nanomolar range (Grun & Blumberg, 2006). Indeed it is the most potent agonist known for these receptors.

1.3.3 Endocrine disruptors produce non-linear responses that can be non-monotonic

Because natural hormones are characterized by non-linear dose-responses, it is expected that endocrine disruptors should also produce non-linear responses. The dose-response curve can take several forms: in its simplest form – a sigmoidal shape – non-linear dose responses occur because hormones act on receptors, which are limited in number and the response itself is "saturable". That means that there is a dose of hormone – or endocrine disruptor – beyond which there is no

further response (see **Figure 1.3A**). However, like hormones, endocrine disruptors can also produce non monotonic dose responses in which the slope of the curve changes sign over the course of the dose-response. For example, when fetal mice are exposed to low or high doses of diethylstilbestrol (DES), a synthetic estrogen, their adult prostate weights are relatively low. However, intermediate doses of DES produced significantly heavier prostates (vom Saal et al., 1997). This also occurs for the female reproductive tract (**Figure 1.5**). A review of bisphenol A (BPA) has shown that there are over 50 reports on non-monotonic dose responses in a variety of tissues [see (Richter et al., 2007) and (Wetherill et al., 2007) for a review of some of these studies and (Alonso-Magdalena et al., 2008; Hugo et al., 2008; Jeng & Watson 2011; Jenkins et al., 2011; Cabaton et al., 2011) for several recent examples]. Indeed non-monotonic dose responses have been reported for more than a dozen natural hormones and more than 60 endocrine disruptors in both cell culture and animal experiments [reviewed in (Vandenberg et al., 2012)]. Recent research also suggests that non-monotonic responses can be extended to the population level. For example, individuals in the highest quartile of environmental exposure to dichlorodiphenyldichloroethane (DDE, an estrogenic metabolite of the pesticide, DDT) have decreased BMI and blood triglyceride levels compared to individuals in the third quartile (Lee et al., 2011). Moreover, women exposed to the lowest and highest doses of dioxin after an industrial accident had no changes to the age at which they entered menopause, although those women exposed to intermediate doses had an increased risk of early menopause (Eskenazi et al., 2005). The actual mechanisms to explain these non-monotonic effects at the population level have not yet been identified, but it is important to recognize that these dose-response characteristics are fully within the realm of hormone action and endocrine disruption; indeed they are to be expected.

1.3.4 Do endocrine disruptors act at low doses?

Hormones act at low doses, in part by virtue of their strong affinity for their receptors (see 1.2.3). Some endocrine disruptors also have a very high affinity for nuclear receptors (e.g. tributyltin for PPARγ), and can act at very low doses primarily as a result. However, it is important to recognize that endocrine disruptors can act at low doses even if their affinity for hormone receptors is considerably lower than that of the native hormone. This can happen, in part, because the impact of small changes in hormone action at the low end of the dose-response curve is much greater than at the high end of the dose-response curve (see **Figure 1.3A**). In addition, differences in receptor abundance have a very large effect on the concentration of hormone (or endocrine disruptor) required to produce an effect (see **Figure 1.3B**). In addition, endocrine disruptors may have different potencies on different receptor isoforms (e.g. ERα or ERβ). Therefore, the "potency" of an endocrine disruptor will be highly dependent upon several important factors. This explains why some cells and tissues – or developmental time points – are much more sensitive to endocrine disruptors than others.

Table 1.4. Examples of EDCs with low dose effects (in animals) (Vandenberg et al., 2012).

Insecticides/Fungicides	Industrial/General
Chlordane	Arachlor 1221
Chlorothalonil	Bisphenol A/Genistein/DES
Chlorpyrifos	Dioxin
DDT	4-methylbenzylidene
Heptachlor	Methylparaben
Hexachlorobenzene	Nicotin
Maneb	Nonphenol
Parathion	Octyphenol
Methoxychlor	Sodium Fluoride
Tributyltin oxide	PBDEs/PCBs
Vinclozolin	Perchlorate

There are many examples of low dose effects of endocrine disruptors (**Table 1.4**), (Vandenberg et al. 2012).

We have focused our discussion on the principles of endocrinology described above and from this perspective, environmental chemicals interacting with endocrine systems can exert effects at low, environmentally relevant doses, and will exhibit dose-response curves that are non-linear and potentially non-monotonic. However, this represents one of two perspectives that are currently under debate. The term "low dose" is defined in two ways. The first is a dose below that which is traditionally accepted by toxicologists as the no adverse effect level (NOAEL; Owens & Chaney, 2005). The second is that of a dose that is environmental relevant to humans, (e.g., Owens & Chaney, 2005). One perspective is that chemicals exert toxicologically relevant adverse effects in a manner that is or approximates linearity; i.e., the dose makes the poison. In addition, the endpoints traditionally captured in toxicological studies are sufficient to determine all adverse outcomes (Owens & Chaney, 2005). In contrast, the "low dose" hypothesis posits that exogenous chemicals that interact with hormone action can do so in a manner that is quite specific such that traditional toxicological endpoints are not sufficient to preclude adverse outcome, and they do so with dose responses that are nonlinear and potentially non-monotonic (Vandenberg et al., 2012).

1.3.5 When do endocrine disruptors act?

Endocrine disruptors can act throughout life just as hormones do by interacting with the same pathways as hormones (see **Table 1.2**). When chemicals with endocrine disrupting activity are present during development, they will affect programming of cell and tissue development and thus their effects are expected to be permanent. When the same endocrine disruptor

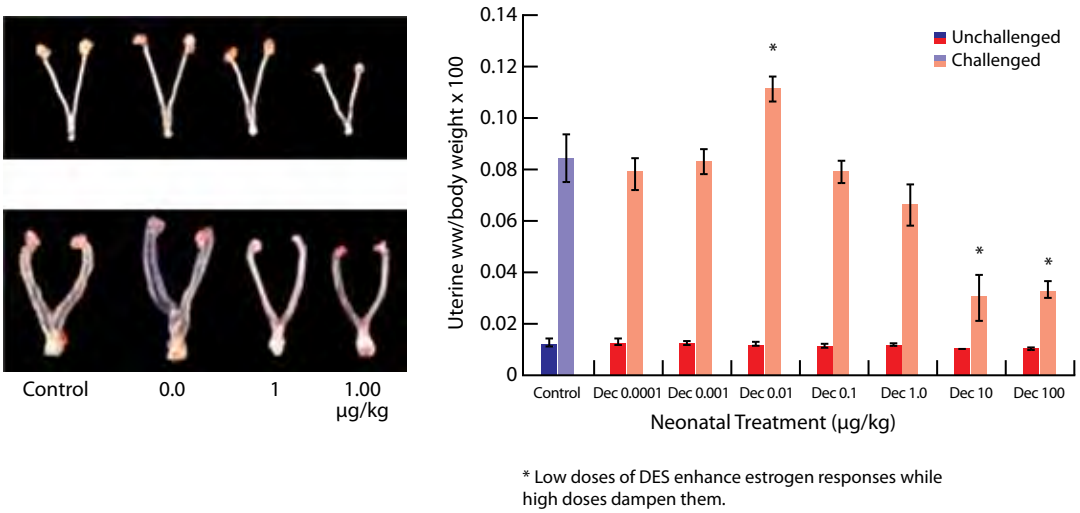

Figure 1.5. Non-monotonic dose response of DES on uterine weight. (Figure from Newbold et al. (2004), redrawn; Used with permission of the publisher).

is present later – in childhood or in the adult – the effects will be different and could be transient. The difference in sensitivity and action of endocrine disruptors over the lifespan has several important implications. First, when studies are designed to link human exposures to specific outcomes, it is important to measure chemical exposures at the developmental time-point that is appropriate for the specific outcome measured. Of course, the outcome may not be visible until adulthood in some cases. This may be more difficult for chemicals that do not persist in the body (e.g. many pesticides), than for chemicals that do (e.g. flame retardants, POPs). Another important implication is that not all endpoints of hormone action will exhibit the same sensitivity to chemical exposures.

The ability of endocrine disruptors to alter the normal hormonal control of development is perhaps the most significant consequence of exposure, because developmental effects will occur at lower doses than are required for effects in adults (Alonso-Magdalena et al., 2010). Additionally, the effects of exposure to endocrine disruptors during development will remain throughout life, due to their effects on programming of cell differentiation and tissue development, resulting in a tissue that has a different predisposition for disease in adulthood to that of a non-exposed tissue. For example, a low dose of bisphenol A during fetal mouse development predisposes the prostate to the cancer-causing actions of low doses of estrogen during adulthood but at birth the prostate "looks" normal and the effect of BPA can only be picked up by an 'omics analysis (Prins et al., 2008). **Table 1.1** shows a list of diseases that have been observed in animal models after acute exposures to endocrine disrupting chemicals during development. This list contains the diseases that were highlighted at the beginning of this chapter as those that have increased in the last few decades. In addition, similar diseases and disorders have been reported in wildlife populations in some cases in relation to exposure to chemical contaminants that are known to be endocrine disruptive. The diseases caused by endocrine disruptors will be described in more detail in Chapter 2.

1.3.6 Endocrine disruptors, the epigenome and transgenerational effects

Parents pass on their genes to their children, and in so doing, pass on various traits associated with those genes. The combination of all genes in a species is referred to as the "genome" and "genomic" studies refer to those studies designed to understand how various patterns of genes are controlled. Cells in the body can pass on heritable traits to their cellular progeny without altering their genome. During development, a single cell – the ovum – will divide, multiply and differentiate and ultimately become an adult. Development from this perspective is a process of "fate restriction" – permanently turning on or turning off different combinations of genes required for a cell to be a functional cell in the liver, kidney, brain, etc. It turns out that this view of development is not complete. Instead it is clear that development is controlled not only by genetics but also by epigenetics, and thus, is subject to changes depending on the "environment". Epigenetics is broadly defined as those heritable changes in the genome not dependent upon changes in genetic sequences (e.g. DNA methylation or histone modification). It is these epigenetic processes that define and control tissue development by controlling gene expression. Thus, a major route by which hormones act during development is by changing the epigenome – the combination of genes that can or cannot be expressed. Though the mechanisms underlying these effects are a relatively new area of study, one manifestation of endocrine disruption is to alter a small subset of hormone-dependent epigenetic mechanisms and thereby alter development. Importantly exogenous chemicals have also been shown to produce heritable "transgenerational"

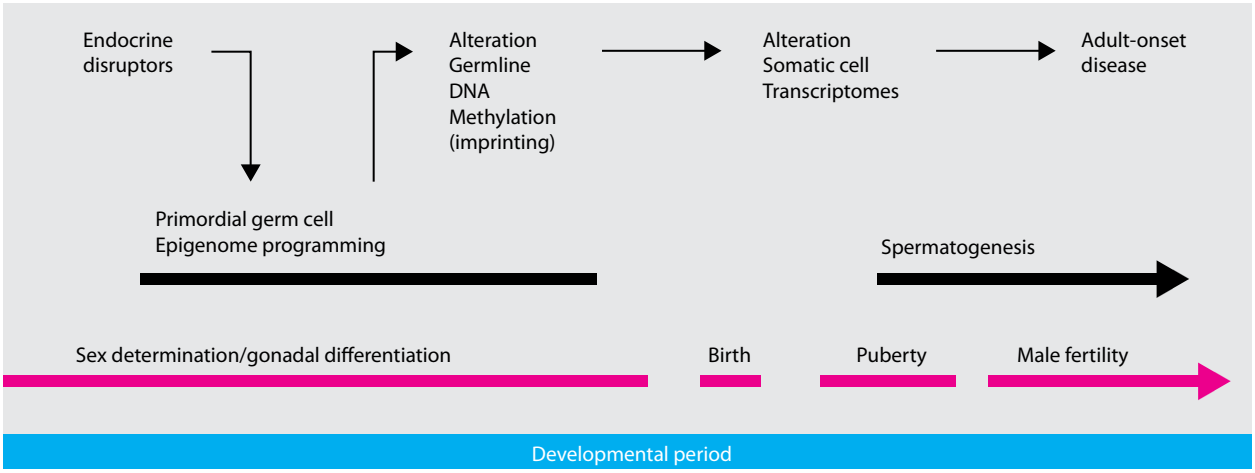

Figure 1.6. Potential mechanism by which EDCs may affect disease transmission across generations. In this scenario, EDC exposure during the period of germ cell programing can alter epigenetic marks which are then transmitted both to germ cells (i.e., gametes) and via an unknown mechanism thereby to future generations as well as in the somatic cells that develop in the embryo thereby altering tissue development. These changes have been shown in animal studies to result in adult disease (Skinner & Guerrero-Bosagna, 2009; Used with publisher's permission).

effects as a result of their ability to alter epigenetic processes. This issue first arose with studies in which an anti-androgenic pesticide (vinclozolin) was given to developing mice at a single time when the testis was in a critical period of development. Vinclozolin produced adverse effects on the developing testis, and this effect was passed on to the following three generations of mice (reviewed in (Skinner, Manikkam & Guerrero-Bosagna, 2011)). This effect is likely to be caused by epigenetic changes that were transmitted with high fidelity from one generation to the next via the germ cells (**Figure 1.6**). A number of exogenous chemicals have now been shown to influence epigenetic mechanisms and to produce effects in several generations of animals. We have a great deal to learn about this issue, but it is plausible that chemical exposures during pregnancy will affect the health of several subsequent generations of people and wildlife that are not themselves exposed.

1.3.7 Evidence for a common mechanism for human/wildlife effects

Estrogen, androgens and thyroid hormones are identical in all vertebrates. However, the receptors are somewhat different among different vertebrate classes and this can influence the ability of exogenous chemicals to interact with them. Endocrine disruptor screening methods for wildlife have been developed using estrogen receptors (ERs) and androgen receptors (ARs) from various animal species, including fish, amphibians, and reptiles (Katsu et al., 2007; 2010). These studies showed species differences in sensitivities of ERs to chemicals. Therefore, in order to protect biodiversity from endocrine disruptors, we need to understand the molecular mechanisms underlying species differences in hormone receptor sensitivity in various wildlife species. Receptor-mediated mechanisms have received the most attention, but other mechanisms (e.g. hormone synthesis, transport and metabolism, activation of nuclear receptors, gene methylation) have been shown to be equally important (IPCS, 2002; Tabb & Blumberg, 2006). For most associations reported between exposure to endocrine disruptors and a variety of biological outcomes, the mechanisms of action are poorly understood.

The endocrine systems of vertebrates largely share molecular mechanisms such as the ability of particular chemicals to bind to steroid receptors (Iguchi & Katsu, 2008). However, the physiological consequences of these mechanisms - for instance, for sex differentiation - differ in different classes of vertebrates. For example, sex is determined by the *sry* gene in mammals (Sinclair et al., 1990), the *dmy* gene in the medaka fish (Matsuda et al., 2002), whereas temperature-dependent sex determination is common in crocodilians and turtles. Estrogen is quite important in the development of ovaries in fish, amphibians, and reptiles, and very likely plays a role in birds as well. Likewise, critical developmental windows of sensitivity - periods when hormonal or xenobiotic chemicals can act during development - differ among vertebrate species.

In insects and crustaceans, reproduction and development are controlled mainly by novel steroids termed ecdysteroids, such as ecdysone and juvenile hormones. The functions of these are well understood in model species of insect (*Drosophila)* and in some aquaculture species of crustacean. For the remaining invertebrate species, information on the endocrine system and the hormone receptor system is limited. The ecdysone receptor has been cloned in *Daphnia magna* (Kato et al., 2007), but no juvenile hormone receptor or binding protein has been identified.

Of the "vertebrate" steroid hormones and their receptors, ER homolog genes have identified in molluscs such as *Aplysia,* octopus, and a marine snail (rock shell; *Thais clavigera*) showed no ligand binding, but they did display ligand-independent gene activation (Thornton, 2003). Thus, functional

nuclear-type ER may not be present in these invertebrates. In annelids, however, a functional ER activated by estradiol has been isolated (Keay & Thornton, 2009) and in rotifers, a membrane progesterone receptor may also be functional (Stout et al., 2010) Membrane ERs have been found in vertebrates and act as an acute response system to estrogens. Therefore, we cannot rule out the possibility that membrane ERs could be present in invertebrate species.

Despite our lack of knowledge on their fundamental endocrinology, chemicals that affect hormonal activities in vertebrates also appear to affect several invertebrate species, such as *Hydra vulgaris,* copepods, barnacles, nematodes, freshwater mud snails, and sea urchins (Fox, 2005). Juvenile hormone agonists used as pesticides induced a reduction of reproduction in parthenogenic *D.magna* and resulted in 100% male offspring (Oda et al., 2005), as would be expected, but the effects of "vertebrate" steroid hormone antagonists and agonists are less clear. In particular, the susceptibility of molluscs to morphological and physiological disruption by estrogenic compounds is a subject of current debate. Amongst the most extreme effects reported are those exerted by bisphenol A (BPA), 4-tert octylphenol (OP) and ethinylestradiol on reproductive output and morphology of the neo-tropical freshwater snail *Marisa cornuarietis,* including increased oocyte production and egg-laying in females and gross morphological effects on the sex organs in both developing juveniles (e.g. formation of additional sex organs in females) and adults (e.g. reduction in male penis length) (Oehlmann et al., 2000, 2006; Schulte-Oehlmann et al., 2004). In direct conflict with these reports are those in which adult M. cornuarietis were exposed to BPA using a different experimental design (Forbes et al., 2007) showing clearly that these effects were not observed. These conflicting reports have fuelled controversy (Dietrich et al., 2006) surrounding the true sensitivity of this species, and molluscs in general, to estrogen mimics and, also, the perceived safety of the aquatic environment from the impacts of these xenestrogens. BPA, in particular, is purported to be much more potent in molluscs than in other aquatic organisms.

Clarifying the molecular basis of the action of hormones and endocrine disruptors on invertebrates is essential to aid in explaining differences in the responses of vertebrates and invertebrates at the cellular and organismal levels and to elucidate the ecological effects of exposure to endocrine disruptors starting at the bottom of the food chain (Iguchi, Watanabe & Katsu, 2006).

1.3.8 Endocrine disruptors and cocktail effects

When the toxicity of chemicals is evaluated, their effects are usually considered in isolation, with assumptions of "tolerable" exposures derived from data about one single chemical. These assumptions break down when exposure is to a large number of additional chemicals that also contribute to the effect in question. This can be illustrated by considering combined actions between estradiol and other chemicals capable of mimicking the hormone's action. For a long time, the risks associated with these "xenestrogens" have been dismissed, with the argument that their potency is too low to make an impact on the actions of estradiol. But it turned out that xenestrogens, combined in sufficient numbers and at concentrations that on their own do not elicit measureable effects, produced substantial estrogenic effects (Silva, Rajapakse & Kortenkamp, 2002), an observation dubbed "something from 'nothing". When mixed together with estradiol, the presence of these xenestrogens at low levels even led to a doubling of the effects of the hormone (Rajapakse, Silva & Kortenkamp, 2002). All these effects could be predicted accurately from the potency of all single components by making the assumption that the xenestrogens acted together without influencing each other's action (the additivity assumption according to the mixture assessment concept of concentration addition).

The above experiments were conducted with a yeast-based reporter gene system for estrogen action, a test system that lends itself to working cost-effectively with the large sample numbers necessary for conducting multi-component mixture experiments. But for a long time it remained untested, whether the principles of mixture toxicology established in such "test tube" experiments would be applicable to more complicated systems, including experimental animals. A break-through was made with the demonstration that multi-component mixtures of estradiol and other xenestrogens induced vitellogenin in fish in a manner that could be predicted from the effects of the single chemicals (Brian et al., 2005). At first, it seemed counter-intuitive to expect that such experiments would be successful, considering the many sources of variation and error inherent in studies with animals, but the empirical evidence showed that this is not the case.

The predictability of combination effects could be demonstrated with systems even more complex than vitellogenin induction in fish. In a developmental toxicity model in the rat, it was shown that combinations of androgen receptor antagonists worked together additively to produce changes in anogenital distance and retained nipples, effects considered to be the hallmarks of disruption of androgen action in fetal life (Hass et al., 2007). In these models, pregnant female rats were dosed throughout gestation and the effects in male offspring monitored. Similar observations were made with combinations of antiandrogens that work by a variety of different mechanisms (Christiansen et al., 2009, Rider et al., 2008). Studies with thyroid disrupting chemicals also showed additive combination effects at low doses (Crofton et al., 2005).

In many of these in vivo experiments with endocrine disruptors, the "something from 'nothing" principle was shown to apply. These findings challenge current regulatory practice. The experimental doses that are used as a basis for deriving health-based exposure standards (e.g. acceptable daily intakes) cannot be considered safe under all circumstances if exposure is to a large number of chemicals that also produce the effect

of interest. These experiments expose an important knowledge gap that currently hampers progress with the risk assessment of endocrine disrupters (and other chemicals). To truly assess the possible health risks that arise from these chemicals, it is necessary to know the full extent of exposures to exogenous chemicals that exert actions on a specific endocrine pathway, together with their potency of effect. But, we are currently far removed even from having fragmentary information about these issues (Kortenkamp & Faust, 2010). Years of mixing cocktails of endocrine disruptors have shown that the combined effects are largely additive and that the effects of multi-component mixtures can be predicted when the potency of its individual components are known (see the review by Kortenkamp, 2007). The challenge ahead is to define what environmentally relevant mixtures of endocrine disruptors are and to assess their effects.

1.3.9 Endocrine disruptors and toxicity testing methods

Man-made chemicals are an important part of modern life. Human and wildlife populations cannot avoid coming into contact with some chemicals employed in food production (plants and meat), in pathogen control (e.g. insecticides), in the production of modern materials (e.g. plastics), or in the built environment (e.g. insulations, flame retardants) (see Chapter 3 for more details). Considering the importance of these chemicals, and their widespread presence in the environment, it is important that strategies are developed to preclude widespread environmental contamination with endocrine disruptors.

Testing strategies currently employed around the world are based on the premise that endocrine disruptors can be evaluated in the same manner as acute toxicants; this implies that tests at high doses will inform us about low-dose exposures, and it also implies that one endpoint of hormone action can effectively act as a surrogate for all endocrine endpoints. However, as we have introduced in this chapter and will develop further in the following chapters, hormone action is quite complex and depends on the developmental stage and the endpoint being evaluated and that endocrine disruptors act like hormones and not general toxicants. Therefore, it is predictable that endocrine disrupting chemicals will exert effects that are also quite complex and that are not captured using strategies designed to detect acute toxicity with a limited range of exposure paradigms and endpoints evaluated. Specifically, endocrine disruptors will produce non-linear dose responses, sometimes including non-monotonic dose-responses, such that high dose toxicity testing will not be sufficient to predict the effects at low doses. In addition, chemicals that interact directly with hormone receptors may not produce effects on endpoints that are routinely employed in toxicity testing in a manner that extrapolate to other disease endpoints or doses. The sensitivity of different endpoints of hormone action is quite variable and will not be captured by a small subset of endpoints captured in general toxicity studies. Finally, the preponderance of toxicity studies have focused on chemicals that interfere with reproductive and thyroid hormones. Little is known about chemicals that can interact with other endocrine systems, and we should not assume that the current assays employed in toxicity testing will identify those chemicals. Considering this, we cannot be confident that the current system of protecting human and wildlife population from chemicals with endocrine activity is working as well as it should to help prevent adverse health impacts on human and wildlife populations.

It is important to recognize that the identification of human or wildlife health effects of chemical exposures in epidemiological studies is an indication that the pre-market evaluation of chemical effects failed to accurately predict their toxicity. A clear example of this is that of polychlorinated biphenyls (PCBs). These chemicals were produced heavily during the first half of the 20th century and their production was banned in the 1970s because of their potential carcinogenicity and because of their persistence in the environment. However, a large number of studies have now shown that prenatal exposure to these chemicals – even several decades after their production was banned – produces adverse effects on cognitive function in children (Schantz, Widholm & Rice, 2003). Experimental studies have demonstrated the different kinds of effects these chemicals have on important signaling mechanisms including on thyroid hormone, estrogen and calcium signaling in the brain. Despite this, the current guideline studies for identifying chemicals that interfere with thyroid hormone action would not identify PCBs as anti-thyroid agents. Given the importance of thyroid hormone action during development, and the successful screening program for identifying congenital hypothyroidism (CH) at birth, it is paradoxical that PCBs would be missed by these guideline studies. It is critical then to learn from this experience to avoid committing several generations of children to exposures to chemicals that limit their potential (Suvorov & Takser 2008).

1.3.10 Framework for evaluation of evidence for endocrine disruption in humans and wildlife

It is important to use a systematic and transparent approach to evaluating the scientific evidence about the relationship between environmental exposure and health effects. Often, this approach is referred to as weight of evidence (WOE), which is an approach used to characterize the extent to which the available data support the hypothesis that there is a relationship between an exposure and adverse health effect. Various WOE methods have been developed; these methods are often a qualitative process in which reviewers put various strands of evidence together to evaluate whether they are likely to support, or not some relevant associations which can include causality. Descriptors are used to characterize the overall conclusions. For example, IARC uses the terms “sufficient”, “limited”, and “inadequate”, to characterize the evidence related to carcinogenicity (IARC, 2006). Similarly, the US National

Toxicology Program (US NTP) uses the terms "sufficient", "limited" and "insufficient" to characterize the evidence (NTP, 2011). The degree of subjectivity embedded in the WOE process has led to much discussion and there are currently no internationally agreed upon methods with which to perform this process, although many proposals of how to do this exist (see the review in Kortenkamp et al., 2011). One such proposal was presented in the 2002 IPCS document (Chapter 7). Others include Conrad & Becker (2011), Woodruff & Sutton (2011; 2010) Linkov et al., 2009, ANSES (2011), and SCENIHR (2012).

WOE of evidence evaluations, and current systematic review approaches generally focus on an individual chemical exposure and a specific outcome. In contrast, WOE evaluations have not been adapted to answer the broad, more open-ended questions addressed in this report, such as "Is there evidence of endocrine disruption in wildlife?") (EFSA, 2010). In this context, narrative reviews can serve an important first step in compiling scientific evidence that addresses critical questions.

The European Environment Agency has stated that methods for evaluating evidence need to be modified to reflect reality more clearly, by including consideration of multicausality, thresholds, timing of dose, mixtures and delayed negative impacts, especially when evaluating EDCs (Gee 2006; 2008). Similarly for temporality, which says that the putative cause *X* of harm *Y* must come before *Y* appears. This is not robust in a multicausal, complex world of common biological endpoints that can have several biological pathways leading to an adverse health effect. For example, falling sperm counts can have multiple, co-risk factors and resulting overall sperm count trends could be rising, falling, or static, depending on the combined direction and strengths of the co-risk factors and the time lags of their impacts" (Gee, 2006). Chlorine chemicals may or may not be co-risk factors in falling sperm counts, but the previous use of the temporality argument in the IPCS (2002) document does not provide robust evidence that they are not a contributing factor (Gee, 2006; EEA, 2012).

Finally, the evaluation of the strength of the evidence is not the same as the strength of the recommendation. Specifically, different strengths of evidence can be appropriate to justify action to reduce exposures, or other measures, in specific cases depending on their circumstances including the cost of being wrong in both direction (i.e., in acting or not acting). This is analogous to" iatrogenic risk in medicine (risk "caused by the doctor") (NAS, 2009). In the same way that a delay in diagnosis by a physician can increase risk to the patient, delays in the process of assessing risks may increase overall exposure to risk when decisions are delayed" (NAS, 2009). Hill (1965) recognised the case of specificity of different strengths of evidence when deciding whether to act on the evidence, or not, observing "*that, it almost inevitably leads us to introduce differential standards before we convict. Thus on relatively slight evidence we might decide to restrict the use of a drug for early-morning sickness in pregnant women. If we are wrong in deducing causation from association no great harm will be done. The good lady and the pharmaceutical industry will doubtless survive. On fair evidence we might take action on what appears to be an occupational hazard, e.g. we might change from a probably carcinogenic oil to a non-carcinogenic oil in a limited environment and without too much injustice if we are wrong. But we should need very strong evidence before we made people burn a fuel in their homes that they do not like or stop smoking the cigarettes and eating the fats and sugar that they do like."* Indeed, Bradford Hill himself recognised the shortcomings of his framework, stating that *"none of my nine viewpoints can bring indisputable evidence for or against the cause and effect hypothesis and none can be required as a sin qua non……what they can do is help us to make up our minds on the answer to the fundamental question – is there another way of explaining the set of facts before us".* (Hill, 1965).

In the example above, Bradford Hill recognized the need to separate strengths of evidence for causality from strengths of recommended actions, an approach subsequently taken up by the GRADE scheme in clinical medicine, which also recognises this separation (GRADE 2011). As noted by GRADE, "*Not all grading systems separate decisions regarding the quality of evidence from the strength of the recommendations. Those who fail to do so create confusion. High quality evidence doesn't necessarily imply strong recommendations, and strong recommendations can arise from low quality evidence*". (Guyatt et al., 2008). These considerations were not taken into account in the IPCS (2002) document. Further, the use of the Bradford Hill criteria was intended to evaluate evidence with only a few examples given in Tables 7.1 and 7.2 of that document and it was not a suggestion for a general way forward.

In the current document, features specific to endocrine disrupting chemicals were considered. These have been articulated by the Endocrine Society (Zoeller et al., 2012). Their perspective emphasizes the non-linearity of hormone action, the temporal and spatial specificity of hormone action, and the myriad of known ways in which chemicals can interfere with hormone action to produce adverse outcomes. Inherent in any approach employing endocrine principles to assess WOE is a careful evaluation of the science itself, rather than a simple "count" of negative and positive findings for a certain chemical. Approaches have been developed in clinical medicine that applies a systematic, transparent approach to evaluating evidence that provides an overall evaluation of the evidence (similar to WOE approaches in environmental health). Further, more recent development of these approaches (GRADE) allows for both assessing the strength of evidence and strength of recommendations separately to allow decision-making even when scientific evidence is uncertain. While these approaches have been developed for clinical medicine and evaluating randomized control trials, they are not currently amenable to evaluating EDCs, as they do not account for evidence from ecological and toxicology literature and are still evolving in relationship to observational epidemiology studies – all evidence that is critical to environmental health. While these approaches are being developed and tested (Woodruff

& Sutton, 2011), and particularly their application to EDCs, conventional narrative.

A central issue common to all WOE approaches is that the reliability of the data must be evaluated. This, in itself, is a very difficult issue and different authors propose different standards. For example, Conrad & Becker (2011) propose that the use of Good Laboratory Practice (GLP) is an important – if not the essential – element of evaluating laboratory studies. This, however, eliminates almost all modern scientific studies and it seems unreasonable to employ state-of-the-art (non GLP) science to guide public health protection in some domains (e.g. clinical), but not in chemical safety. Many of the standardized protocols carried out to GLP standards are no guarantee of quality if they do not include the most sensitive relative endpoints or exposures during critical windows in development. In contrast, Woodruff & Sutton (2011) and ANSES (2011) propose ways of evaluating the strength of evidence based on scientific standards, independent of the method applied. Experience in clinical medicine with systematic reviews suggests that rigorous, empirically tested evaluation of the methods and study quality will provide the least biased evaluation.

It is also noteworthy that there is no universally accepted scheme for the classification of the results of a WOE assessment for endocrine disruptors (e.g. probable, possible or unlikely), like those developed for carcinogens (by IARC) or for air pollution (by WHO) or for Climate Change (by IPCC). It is also not straightforward to adopt the approaches worked out for carcinogenic modes of action under the auspices of WHO IPCS (Sonich-Mullin et al., 2001, Boobis et al., 2006) for application to endocrine disrupting chemicals. Uniquely, WOE approaches for endocrine disruptors will have to deal with the issues of adversity and mode-of-action at the same time which is currently without precedent. Such methods will have to be elaborated for endocrine disruptors, as recommended by Kortenkamp et al. (2011).

With this in mind, Chapter 2 takes a narrative review of the state of the science on endocrine disruption in humans and wildlife, considering each part of the endocrine system in turn. Emphasis was placed on the literature that appeared after 2000, with a cut-off data by March 2012. Best professional judgment was used to make expert assessments of the data linking exposure to chemicals with each disease/dysfunction. The literature on disease and disorder trends was aggregated, biological plausibility, relevant exposures (considering possible multiple exposures), consistency of the data across species (where endocrinology is similar), dose responses and temporality (considering possible latent effects and multi-causality). Wherever relevant, evidence was integrated from human and ecotoxicological species. The reviewers of the evidence were the expert authors of each of the sub-chapters. Their reviews were then peer-reviewed by an external panel of experts carefully chosen for their knowledge of each of the relevant areas. Both non-GLP and GLP studies were included as long as they were considered to be reliable and relevant.

Concerns about the hazards and risks following widespread exposures to EDCs arise from what is known of the role of hormones in organizing critical events during early development. Consequently, evidence was assessed from a viewpoint that is sensitive to the evidence that EDCs may exert low dose effects and effects during critical windows of susceptibility. Although debates relating to low dose phenomena are likely to go on for the foreseeable future, authoritative bodies (such as the US National Academy of Sciences) have proposed that given multiple chemical exposures and variability in response, that, like for carcinogens, a “threshold” should not be assumed (NAS, 2009). Rather, it is more likely that risks increase linearly also in the low dose range, but this will be difficult to prove because methods for detecting effects at low doses are insufficiently sensitive to observe these thresholds, if they exist. Moreover, because in almost all situations, pre-existing endogenous hormone levels exist, any additional exposure will increase this load in a threshold-independent manner.

Statements of the strength of the evidence are generic (e.g. sufficient or insufficient, strong, moderate or weak). Characterizing the strength of evidence is an integral step in decision-making; however, equally important is to characterize the other aspects critical to decision-making including values and preferences, and consequences of different choices. As pointed out by Bradford Hill and articulated here, weak evidence might be sufficient if there was a possible teratogenic effect of a pregnancy pill prescribed to millions of women worldwide whilst stronger evidence might be needed if there was a probable carcinogen in the workplace. Our goal was that these descriptors provide guidance seeking the goals of protecting wildlife and human health from hormonally active chemicals; however we suggest that a methodology and framework for evaluating evidence for endocrine disruption is further developed together with guidance for future directions for applying transparent, consistent and systematic concepts and terminology on the nature of the EDC-effect relationships.

1.4 Main messages

- Endocrine disruptors are exogenous chemicals or chemical mixtures that can interfere with any aspect of hormone action.
- Endocrine disruptors can act directly on hormone receptors or can act directly on any number of proteins that control the delivery of a hormone to its normal target cell or tissues.
- The affinity of an endocrine disruptor for a hormone receptor is not equivalent to its potency. Chemical potency on a hormone system is dependent upon many factors including receptor abundance.
- Endocrine disruptors produce nonlinear dose responses both in vitro and in vivo; these non linear dose responses can be quite complex and often include non-monotonic dose responses. They can be due to a variety of mechanisms; because endogenous hormone levels fluctuate, no threshold can be assumed.

- Endocrine disruptors show tissue specific effects.
- Endocrine disruptors can act on membrane or nuclear receptors.
- Environmental chemicals can exert endocrine disruptor activity on more than estrogen, androgen and thyroid hormone action. Some are known to interact with multiple hormone receptors simultaneously.
- Sensitivity to endocrine disruption is highest during tissue development; developmental effects will occur at lower doses than are required for effects in adults. Endocrine disruptors can work together to produce combination effects when combined at low doses. The extent of combination effects is governed by the sheer number of endocrine disruptors and their individual potency.
- Testing for endocrine disruption must encompass the developmental period and include lifelong follow-up to assess latent effects.
- Not all endpoints of hormone action will exhibit the same sensitivity to chemical exposures (example, uterine response to BPA).
- Endocrine disruption represents a special form of toxicity, and this must be taken into consideration when interpreting the results of studies of endocrine disrupting chemicals, or when designing studies to clarify the effects of endocrine disrupting chemicals and quantifying the risks to human and wildlife health.
- Endocrine disruptors will exert effects that are also quite complex and that are not captured using strategies designed to detect acute toxicity.
- Efforts are needed to develop systematic and transparent approaches to identify, evaluating and synthesizing the scientific evidence for endocrine disruptors that consider the science of endocrine action.

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Chapter 2

Evidence for endocrine disruption in humans and wildlife

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2.0 Introduction

At the time of the publication of the Global Assessment of the State-of-the-science of Endocrine Disruptors (IPCS, 2002), there was evidence that the health of some wildlife populations had been adversely affected by exposure to EDCs, but very weak evidence that human health was similarly affected. This was mainly due to an insufficient number of rigorous studies carried out on this issue, rather than to a lack of likelihood that EDCs could cause health effects in human populations. There were multiple experimental animal studies showing chemicals could interfere with hormone production and/or action and that this could result in adverse effects. Abnormalities in male reproductive health and development in fish and other wildlife species exposed to contaminants were the early leads for this research.

Over the last decade, scientific understanding of the relationship between the environment and health has advanced rapidly. Of greatest significance is that we now know that there are particularly vulnerable periods during fetal and postnatal life when EDCs alone, or in mixtures, have strong and often irreversible effects on developing organs, whereas exposure of adults causes lesser or no effects. Consequently, there is now a growing probability that maternal, fetal and childhood exposure to chemical pollutants play a larger role in the etiology of many endocrine diseases and disorders of the thyroid, immune, digestive, cardiovascular, reproductive and metabolic systems (including childhood obesity and diabetes) than previously thought possible.

Many of the internationally agreed upon and validated test methods for the identification of EDCs address only a very limited range of the known spectrum of EDC effects. For many effects, adequate test methods do not exist. This introduces uncertainties with potentially serious consequences for human and wildlife populations and also suggests a failure in environmental protection that should be addressed. Although there are genetic, anatomical and physiological constraints, large parts of the endocrine system are highly conserved among vertebrates and, therefore, wildlife should be considered effective and important sentinels of human health. Moreover, the protection of wildlife is vital in ensuring the conservation of biodiversity and safeguarding the important role that biological communities play in preserving ecosystem services and ecological sustainability.

The aim of this chapter is to provide a critical review of the evidence for endocrine disruption in wildlife and humans and to highlight the gaps in knowledge and in chemical test methods that need to be filled if we are to protect humans and wildlife from potential effects of endocrine disrupting chemicals. The focus is on identification of the characteristics of the hazards posed by endocrine disruptors rather than risk assessment. As risk assessment is largely undertaken on a chemical by chemical basis with little or no account taken of other concurrent exposures, it is not appropriate for assessing the combined effect of mixtures of similarly acting endocrine disruptors described here in. Moreover, human exposure data are limited for most EDCs, making accurate risk assessment difficult or impossible for many chemicals.

Plans to comprehensively evaluate chemicals for their endocrine-disrupting activities are already part of the regulatory agenda in the USA and Europe, however, so it may be possible to count the number of chemicals to be taken into account in a “mixtures risk assessment " in the future.

A brief account is given of the historical case of diethylstilbestrol (DES), a “pharmaceutical endocrine disruptor” that caused a variety of unexpected reproductive disorders and diseases in the sons and daughters of the women who took it during pregnancy. These problems arose because of ignorance of the actions of hormones on the programming of tissue structure and function during fetal life. Although DES was a pharmaceutical drug given at relatively high doses, this case study illustrates the spectrum of possible effects that endocrine disrupting chemicals could cause when exposures occur at critical times during the early development of an organism.

2.1 An introduction to endocrine disruption of the reproductive system

2.1.1 The diethylstilbestrol case

DES is a potent synthetic estrogen that was originally synthesized in 1938. It was used extensively to treat pregnant women from the 1940s-1970s to prevent miscarriages and other pregnancy complications (Giusti Iwamoto & Hatch, 1995; Bamigboye & Morris, 2003). It was initially given to women with at-risk pregnancies, but ultimately it was also prescribed to women with normal pregnancies to make babies “healthier”. It is still not known how many people were DES-exposed

worldwide. Anyone born or pregnant in the USA between 1938 and 1971, and until the mid-'80s in some European countries (until 1977 in France), could have been exposed. In the USA alone, there were an estimated 10 million people (mothers, daughters and sons). Subsequently, DES was found ineffective in reducing miscarriages. More importantly, it was linked to a rare form of vaginal cancer in a small number (< 0.1%) of adolescent daughters who were exposed to it during in utero development (Herbst, Ulfelder & Poskanzer, 1971). DES was later associated with more frequent benign reproductive problems in ~90-95% of DES-exposed daughters; reproductive tract malformations and dysfunction, miscarriage, preterm delivery, and low birth weight, ectopic pregnancies, and premature labour and births were reported (for review, Giusti, Iwanioto & Hatch, 1995; Bamigboye & Morris, 2003) and there were also effects on DES sons (described fully in section 2.3). Research found that in utero exposure to DES alters the normal programming of gene families that play important roles in reproductive tract differentiation (Pavlova et al., 1994; Taylor, Vanden, Heuvel & Igarashi, 1997; Miller, Dagenhardt & Sassoon, 1998). As a result, women exposed to DES *in utero* were at increased risk of clear cell adenocarcinoma of the vagina and cervix, structural reproductive tract anomalies, infertility, and poor pregnancy outcomes (Schrager & Potter, 2004). Moreover, developmental exposures may have played a role in increased risk of adult onset of fibroids and endometriosis. Two epidemiologic studies evaluated increased risk of uterine fibroids in women who were prenatally exposed to DES. One study using a more sensitive method to detect fibroids found an elevated risk from prenatal DES exposure, whereas a second study did not find an association with fibroids, but did find an association with paraovarian cysts (Baird & Newbold, 2005; Wise et al., 2005). In utero exposure to DES is associated with an 80% increased risk of endometriosis (Missmer 2004).

As DES-daughters age, they are more susceptible to breast cancer than unexposed, age-matched women. DES-exposed daughters > 40 years exhibit a statistically significant increase in risk of developing breast cancer (Hatch et al., 1998; Palmer et al., 2002; Palmer et al., 2006; Troisi, Potishman & Hoover, 2007); this increased risk is more pronounced in DES-women over 50 years old, though this association did not reach statistical significance (Palmer et al., 2006). DES-exposed mothers also have an increased risk of breast cancer (Giusti, Iwanioto & Hatch, 1995). Prenatally DES-exposed sons suffer a range of reproductive tract problems including malformations (urethral abnormalities, epididymal cysts, and undescended testes) and increased genital/ urinary inflammation (Herbst & Bern, 1981; NIH, 1999; CDC, 2003; Titus-Ernstoff et al., 2010).

These observed human effects of DES have been confirmed in numerous animal models, and predict changes found in DES-exposed humans, such as oviductal malformations (Newbold et al., 1983) and increased incidence of uterine fibroids (McLachlan et al., 1977; Newbold et al., 1998; Baird & Newbold 2005; Hoover et al., 2011), particularly in Eker rats genetically predisposed to uterine fibroids (Cook et al., 2005). There are also second-generational effects (Newbold et al., 1998; Newbold et al., 2000) such as increased menstrual irregularities (Titus-Ernstoff et al., 2006) and possibly ovarian cancer (Blatt et al., 2003) in DES grandaughters. Prenatal DES exposure resulted in hormonal imprinting of the developing uterine myometrium in both wild -type and genetically susceptible rats.

Based on a wealth of accumulated scientific information from humans and experimental animals, DES is well-documented to be a "transplacental carcinogen"; it crosses the placenta, reaches the fetus, adversely affects developing tissues/organs, and causes a myriad of problems including cancer (Herbst & Bern, 1981; NIH, 1999; CDC, 2003; Diamanti-Kandarakis et al., 2009; Hoover et al., 2011). DES caused a major medical catastrophe that continues to unfold today.

DES was eventually banned for use during pregnancy, but experimental studies continue to explore mechanism(s) through which DES causes its adverse effects. The murine model using prenatally DES-exposed outbred mice has been particularly successful in duplicating and predicting abnormalities reported in prenatally DES-exposed humans (for reviews see Newbold, 1995; Newbold, 2004). DES taught us the following four important lessons that can guide our investigations on endocrine disrupting chemicals now and in the future. Lessons are further discussed in detail in Chapter 2.43:

1. Exposure to endocrine disruptors during early (fetal) development can induce disorders of the endocrine system in the fetus, whilst the mother may appear healthy.
2. The risk of health impacts from exposure to hormone disruptors is especially high during early development when multiple developing tissues may be affected.
3. An endocrine disease or disorder induced during early development might only be apparent decades later, and exposure to this one chemical could lead to multiple health risks in exposed individuals and in subsequent generations.
4. Since all the effects shown in animal models with DES have also been shown in human situations (indeed animal models even predicted human outcomes), DES is also a good example of the need for extrapolation of animal data on EDCs to humans.

2.1.2 Endocrine disruptors in the reproductive system – experimental results

There is a large body of literature from experimental studies with rodents on the adverse effects of DES and of other EDCs on the reproductive system, examples of which are compiled in **Table 2.1.** Unlike human studies, animal studies enable the investigator to measure all indices of hormone action at various times during development and thus to accurately interpret the relationship between exposure and all of the effects on the

endocrine system. Although most of the chemicals listed would appear to be weaker (less potent) estrogens than DES, their effects can be equally undesirable when the exposure occurs in early development where potency seems less important (Diamanti-Kandarakis et al., 2009). These data present a compelling case for chemically-induced endocrine disruption as one factor in the causation of male and female reproductive disorders and diseases in both human and wildlife populations. The use of some of these chemicals is now prohibited in many countries (e.g. alkylphenols, some of the phthalate plasticizers, PCBs and the pesticide DDT, in addition to DES; see Chapter 3 for a description of these chemicals), but others are still in wide use. Moreover, we now recognize that an increasing number of chemicals in modern commerce can interfere with male and female reproductive function (see Chapter 2.2 & 2.3 for a current review of effects).

There are currently many gaps in the available chemical test methods for screening chemicals for endocrine disrupting effects. Regulatory tests for many wildlife taxa are currently not developed and of the mammalian assays available, most do not cover endocrine endpoints adequately enough to detect the effects of endocrine disrupting chemicals described in this chapter. Perhaps most importantly, the exposure periods do not cover critical developmental windows of increased susceptibility now known to exist. Delayed effects that can manifest themselves with ageing are not included either.

Table 2.1. Effects of endocrine disruptors observed in the reproductive system of animals (adapted from WHO, 2012).

Contaminant	Sex	Observation	References
Diethylstilbestrol (DES)	Male	Sterility	McLachlan, 1977
		Epididymal cysts	McLachlan, 1977
		Cryptorchidism	McLachlan, 1977
		Reduction in testis weight	Fisher et al., 1999; Lewis et al., 2003; McKinnell et al., 2001
		Testicular lesions	McLachlan, 1977
		Inflammatory disease of the accessory sex glands	McLachlan, 1977
		Reduction in the number of spermatogonia with multinucleate cells in lumina of testis	McLachlan, 1977
		Nodular enlargements of the seminal vesicles and/or prostate	McLachlan, 1977
		Distension and overgrowth of the rete testis	Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002
		Distension and reduction in epithelial height of the efferent ducts	Fisher et al., 1999; McKinnell et al., 2001; Rivas et al., 2002
		Underdevelopment of the epididymal duct epithelium	McKinnell et al., 2001
		Reduction in epithelial height in the vas deferens	McKinnell et al., 2001; Rivas et al., 2002
		Convolution of the extra-epididymal vas	McKinnell et al., 2001;
		Decreased testosterone levels	Rivas et al., 2002; Yamamoto et al., 2003
		Increased gonadotrophin levels	Yamamoto et al., 2003
		Decreased AR expression in testis, epithelium of the rete testis, caput and cauda epididymis and vas deferens	McKinnell et al., 2001
	Female	Decrease in reproductive capacity	McLachlan, 1977
		Impaired ovarian function	McLachlan, 1977
		Increased uterus weight	Lewis et al., 2003
		Squamous metaplasia in the oviducts, uterus and cervix	McLachlan, 1977
		Increased the size of sexually dimorphic nucleus of the preoptic area	Faber & Hughes 1991; Lewis et al., 2003
		Cystic hyperplasia of the endometrium and uterine adenocarcinoma	McLachlan, 1977
		Epidermoid tumours of the cervix and vagina	McLachlan, 1977
		Glandular elements and cellular atypia in the vaginal epithelium	McLachlan, 1977
		Advanced development of primary and secondary follicles in the ovary	Yamamoto et al., 2003
		Decreased pituitary responsiveness to GnRH	Faber & Hughes 1991
		Increased pubertal FSH levels	Yamamoto et al., 2003
Tributyltin	Male	Increased anogenital distance	Adeeko et al., 2003
		Reduced the number of Sertoli cells and gonocytes in fetal testis	Kishta et al., 2007
	Female	Reduced the number of germ cells in fetal ovaries	Kishta et al., 2007
		Increased post-implantation loss	Adeeko et al., 2003

Table 2.1. (Continued)

Contaminant	Sex	Observation	References
Phytestrogens (Genistein, Daidzein)	Male	Impaired erectile function	Pan et al., 2008
		Decreased plasma testosterone levels	Pan et al., 2008
		Increased testis weight	Fisher et al., 1999
		Reduction in epithelial height of the efferent ducts	Fisher et al., 1999
		Increased pituitary response to GnRH	Faber & Hughes, 1991
	Female	Decreased pituitary responsiveness to GnRH	Faber & Hughes, 1991
		Increased the size of sexually dimorphic nucleus of the preoptic area	Faber & Hughes, 1991; Lewis et al., 2003
		Increased the weight of uterus	Lewis et al., 2003
		Decreased the weight of uterus	Awoniyi et al., 1998
		Decreased the weight of ovaries	Awoniyi et al., 1998
		Reduced serum estradiol levels	Awoniyi et al., 1998
		Reduced serum progesterone levels	Awoniyi et al., 1998; Lewis et al., 2003
		Irregular estrous cycle	Nagao et al., 2001
		Histopathological changes in the ovaries and uterus	Nagao et al., 2001
		Induced permanent estrous	Lewis et al., 2003
		Decreased the age of vaginal opening	Lewis et al., 2003
Alkyl phenol ethoxylates (*p-tert*-octylphenol, *p*-nonylphenol)	Male	Increased testis weight	Fisher et al., 1999
		Decreased testis weight	de Jager, Bornman & Oosthuizen, 1999; Pocock et al., 2002
		Decreased seminiferous tubule diameter	de Jager, Bornman & Oosthuizen, 1999; Pocock et al., 2002
		Decreased epididymal weight	de Jager, Bornman & Oosthuizen, 1999
		Decreased total cauda epididymal sperm count	de Jager, Bornman & Oosthuizen, 1999
		Reduction in epithelial height of the efferent ducts	Fisher et al., 1999
	Female	Post-implantation embryonic loss	Harazono & Ema, 2001
		Irregular estrous cycle	Katsuda et al., 2000; Pocock et al., 2002
Alkylphenolethoxylates (*p-tert*-octylphenol, *p*-nonylphenol)	Female	Increased sexual motivation towards a female teaser	Pocock et al., 2002
		Decreased the weight of ovaries	Pocock et al., 2002
		Increased the size of sexually dimorphic nucleus of the preoptic area	Herath et al., 2001
		Decreased the age of vaginal opening	Katsuda et al., 2000
		Persistent estrus	Katsuda et al., 2000
		Increased relative uterine weight	Katsuda et al., 2000
		Decreased serum gonadotropin levels	Katsuda et al., 2000
		Decreased serum progesterone levels	Katsuda et al., 2000
		Increased serum inhibin levels	Katsuda et al., 2000

Table 2.1. (Continued)

Contaminant	Sex	Observation	References
Phthalate esters (DEHP, BBP, DiNP, DBP)	Male	Nipple retention	Barlow, McIntyre & Foster, 2004; Borch et al., 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Decreased testis weight	Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000
		Reduced anogenital distance	Borch et al., 2004; Barlow, McIntyre & Foster, 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000
		Cryptorchidism	Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Reduced accessory sex organ weights	Andrade et al., 2006; Barlow, McIntyre & Foster, 2004; Gray et al.,1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Lesion of the rete testis	Barlow, McIntyre & Foster, 2004
		Hemorrhagic testis	Gray et al., 1999b; Gray et al., 2000
		Cleft phallus and hypospadias	Barlow, McIntyre & Foster 2004; Gray et al., 1999b; Gray et al., 2000; Mylchreest et al., 1999; Mylchreest et al., 2000
		Multinucleated gonocytes	Gray et al., 2000; Parks et al., 2000
		Agenesis of the seminal vesicles and coagulating glands	Gray et al., 2000; Mylchreest et al., 2000
		Agenesis of bulbourethal glands	Gray et al., 2000
		Agenesis of ventral prostate	Barlow, McIntyre & Foster 2004; Gray et al., 2000
		Agenesis of gubernacular cords	Gray et al., 2000
		Agenesis of epididymis and vas deferens	Barlow, McIntyre & Foster, 2004; Gray et al., 1999b; Mylchreest et al., 1999; Mylchreest et al., 2000
		Histopathological changes of testis	Barlow, McIntyre & Foster, 2004; Mylchreest et al., 1999; Mylchreest et al., 2000; Parks et al., 2000
		Delayed preputial separation	Gray et al., 1999b; Mylchreest et al., 1999
		Reduced fertility	Gray et al., 1999b
		Reduced fecundity	Gray et al., 1999b
		Reduced cauda epididymal sperm numbers	Gray et al., 1999b
		Reduced daily sperm production	Andrade et al., 2006
		Reduced plasma and/or testicular testosterone levels	Borch et al., 2004; Parks et al., 2000
		Increased serum testosterone levels	Andrade et al., 2006
		Reduced serum inhibin B levels	Borch et al., 2004
		Increase plasma LH levels	Borch et al., 2004; Grande et al., 2007
	Female	Uterine abnormalities	Gray et al., 1999b
		Reduced fertility	Gray et al., 1999b
Chlorinated pesticides (DDE [1])	Male	Nipple retention	Gray et al., 1999b; Kelce et al., 1995; You et al., 1998
		Hypospadias	Gray et al., 1999b
		Reduced accessory sex organ weights	Gray et al.,1999b; Kelce et al., 1995
		Reduced anogenital distance	Kelce et al., 1995; You et al., 1998
		Delayed preputial separation	Kelce et al., 1995
		Abnormally small penis	Guillette et al., 1994
		Poorly organized testis	Guillette et al., 1994
		Decreased plasma testosterone levels	Guillette et al., 1994
	Female	Increased plasma estradiol levels	Guillette et al., 1994
		Abnormal ovarian morphology with large number of polyovular follicles and polynuclear oocytes	Guillette et al., 1994

Table 2.1. (Continued)

Contaminant	Sex	Observation	References
Dioxins	Male	Reduced accessory sex organ weights	Gray et al., 1995; Mably et al., 1992a; Mably et al., 1992b; Ohsako et al., 2001; Simanainen et al., 2004
		Decreased testis weight	Gray et al., 1995; Mably et al., 1992b
		Delayed preputial separation	Gray et al., 1995a
		Reduced anogenital distance	Gray et al., 1995; Mably et al., 1992a; Ohsako et al., 2001; Simanainen et al., 2004
		Delayed testis descent	Mably et al., 1992a
		Epididymal malformations	Gray et al., 1995; Simanainen et al., 2004
		Altered sex behaviour	Gray et al., 1995
		Decreased sperm numbers	Gray et al., 1995; Mably, Moore & Peterson 1992b; Simanainen et al., 2004
Dioxins	Male	Decreased daily sperm production	Mably, Moore & Peterson, 1992b
		Dose-related tendencies to decrease plasma testosterone and DHT	Mably et al., 1992a
	Female	Delayed puberty	Gray et al., 1995
		Clef phallus	Gray et al., 1995
		Vaginal thread	Gray et al., 1995
		Reduced ovarian weight	Gray et al., 1995
		Enhanced incidences of constant estrus	Gray et al., 1995
		Cystic endometrial hyperplasia	Gray et al., 1995
		Decreased fertility rate	Gray et al., 1995
		Reduced fecundity	Gray et al., 1995
Polychlorinated biphenyls (PCBs; PCB 77, 118, 126, 132, 169)	Male	Reduced accessory sex organ weights	Faqi et al., 1998; Gray et al., 1999b; Hsu et al., 2007; Kuriyama & Chahoud, 2004
		Decreased testis weight	Gray et al., 1999b; Kuriyama & Chahoud, 2004
		Increased testis weight	Faqi et al., 1998
		Increased epididymis weight	Faqi et al., 1998
		Reduced anogenital distance	Faqi et al., 1998
		Increased anogenital distance	Kuriyama & Chahoud, 2004
		Delay in onset of spermatogenesis, preputial separation and sex accessory growth	Gray et al., 1999b
		Decreased sperm number and total motile sperm count	Gray et al., 1999b; Hsu et al., 2007; Kuriyama & Chahoud, 2004
		Increased daily sperm production	Faqi et al., 1998
		Decreased serum testosterone levels	Faqi et al., 1998
		Increased the number of abnormal sperm	Kuriyama & Chahoud, 2004
		Altered sex behaviour	Faqi et al., 1998
	Female	Vaginal thread	Gray et al., 1999b
		Mild hypospadias Delayed the timing of vaginal opening	Gray et al., 1999b Faqi et al., 1998
Dicarboximide Fungicides (Vinclozolin, Procymidone)	Male	Hypospadias with cleft phallus	Gray, Ostby & Kelce 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Reduced anogenital distance	Cowin et al., 2010; Elzeinova et al., 2008; Gray, Ostby & Kelce 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Decreased testis weight	Elzeinova et al., 2008; Hellwig et al., 2000
		Cryptorchidism	Gray, Ostby & Kelce, 1994; Hellwig et al., 2000; Ostby et al., 1999

Table 2.1. (Continued)

Contaminant	Sex	Observation	References
Dicarboximide Fungicides (Vinclozolin, Procymidone)	Male	Increased the number of apoptotic germ cells in testis	Cowin et al., 2010
		Nipple retention	Gray, Ostby & Kelce, 1994; Gray et al., 1999a; Hellwig et al., 2000; Ostby et al., 1999
		Reduced accessory sex organ weights	Cowin et al., 2010; Elzeinova et al., 2008; Gray, Ostby & Kelce, 1994; Gray et al., 1999a; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Glandular atrophy and chronic inflammation of prostate	Cowin et al., 2010; Gray et al., 1999b; Hellwig et al., 2000; Ostby et al., 1999
		Reduced secretion and chronic inflammation of seminal vesicles	Hellwig et al., 2000
		Epididymal granulomas	Gray, Ostby & Kelce, 1994; Gray et al., 1999a; Ostby et al., 1999
		Chronic inflammation of epididymis	Hellwig et al., 2000
		Agenesis of prostate	Gray, Ostby & Kelce, 1994
		Spermatogenic granuloma	Hellwig et al., 2000
		Decreased sperm number and daily sperm production	Elzeinova et al., 2008; Gray et al., 1994; Gray et al., 1999a
		Increased sperm head abnormalities	Elzeinova et al., 2008
		Reduced elongated spermatid content per testis	Cowin et al., 2010
		Low ejaculated sperm count	Gray et al., 1999a
		Abnormal morphology of seminiferous tubules	Elzeinova et al., 2008; Gray, Ostby & Kelce 1994
		Decreased fertility	Gray, Ostby & Kelce, 1994
		Reduction of erections during the ex copula penile reflex test	Colbert et al., 2005
		Increase in seminal emissions during the ex copula penile reflex tests	Colbert et al., 2005
		Decreased serum testosterone levels	Gray, Ostby & Kelce, 1994
Herbicides (Linuron)	Male	Nipple retention	Gray et al., 1999b
		Reduced accessory sex organ weights	Gray et al., 1999b
		Delayed preputial separation	Gray et al., 1999b
		Decreased testis weight	Gray et al., 1999b
		Reduced spermatid number	Gray et al., 1999b
		Decreased anogenital distance	Gray et al., 1999b
		Epispadias	Gray et al., 1999b
		Testicular and epididymal malformations	Gray et al., 1999b
Lead	Male	Reduced accessory sex organ weights	Ronis et al., 1996
		Decreased testis weight	Ronis et al., 1996
		Enlarged prostate weight	McGivern, Sokol & Berman, 1991
		Reduced serum testosterone levels	Ronis et al., 1996
		Decreased sperm counts	
		Reduced serum LH levels	Ronis et al., 1996
		Reduced volume of the sexually dimorphic nucleus of the preoptic area	McGivern, Sokol & Berman, 1991
		Less masculine sex behaviour	McGivern, Sokol & Berman, 1991
		Irregular release pattern of gonadotropins	McGivern, Sokol & Berman, 1991
	Female	Delayed the timing of vaginal opening and the day of first diestrus	Dearth et al., 2002; Kimmel et al., 1980; McGivern, Sokol & Berman, 1991; Ronis et al., 1996
		Prolonged and irregular periods of dioestrus	McGivern, Sokol & Berman, 1991;
		Disruption of estrous cycling	Ronis et al., 1996
		Suppressed serum levels of IGF-I, LH and/or oestradiol	Dearth et al., 2002; Ronis et al., 1996
		Irregular release pattern of gonadotropins	McGivern, Sokol & Berman, 1991

Table 2.1. (Continued)

Contaminant	Sex	Observation	References
Cadmium	Male	Time- and dose-dependent decrease in sperm motility	Benoff et al., 2008
		Partial or entire evacuation of the seminiferous tubules	Toman, Massanyi & Uhrin, 2002
		Increased the diameter of seminiferous tubules	Toman, Massanyi & Uhrin, 2002
		Reduced epithelial volume and increased lumen of tubule in the epididymis	Toman, Massanyi & Uhrin, 2002
		Hyperemic testes with extensive haemorrhaging, destruction of all of the presperm spermatogenic cells, and general necrosis and shrinkage of the seminiferous tubules	Foote, 1999
		Decrease in sperm output	Foote, 1999
		Reduced size of the testis	Tam & Liu, 1985
		Reduced number of differentiating germ cells in 16.5-day embryos	Tam & Liu, 1985
		Spermatozoa had poor ability to capacitate in vitro and showed a low fertilizing capability	Tam & Liu, 1985
	Female	Perturbed estrous cycles	Ishitobi & Watanabe, 2005
		Reduced number of differentiating germ cells and the size the ovary in 16.5-day embryos	Tam and Liu, 1985
		Tendency towards delayed timing of vaginal opening	Ishitobi & Watanabe, 2005
		Earlier onset of vaginal opening	Johnson et al., 2003
		Increased the epithelial area and the number of terminal end buds in the mammary glands and decreased the number of alveolar buds	Johnson et al., 2003
Manganese	Male	Increased serum gonadotrophin levels	Lee et al., 2006
		Increased serum testosterone levels	Lee et al., 2006
		Increased daily sperm production and efficiency of spermatogenesis	Lee et al., 2006
	Female	Increased serum gonadotropin levels	Pine et al., 2005
		Increased serum estradiol levels	Pine et al., 2005
		Earlier onset of vaginal opening	Pine et al., 2005

[1] Human health aspects of indoor spraying of DDT have been extensivly reviewed in IPCS, 2011. The document includes chapters on endocrine and reproductive effects, neurological effects and cancer.

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2.2 Endocrine disrupting chemicals and female reproductive health

2.2.1 Overview of female reproductive health trends in humans and wildlife and evidence for endocrine disruption

The knowledge gained from the case of the synthetic estrogen, diethylstilbestrol, can now be used to protect humans and wildlife from the effects of endocrine disrupting chemicals in widespread use. Given that endogenous estrogens participate in female reproductive development and function, it is biologically plausible that exposures to endocrine disrupting chemicals are influencing female reproductive health. If they are, then it is possible that females today could be suffering from higher rates of reproductive problems than their ancestors did. Whilst historical data that could definitively answer this question do not exist, currently-available data in human populations from all countries that have been studied show that millions of women are today affected by the following reproductive disorders:

- ***Polycystic ovary syndrome (PCOS) can affect 3 to 15% of women of reproductive age*** (Teede, Deaks & Moran, 2010; Broekmans et al., 2006). PCOS is the leading cause of sub-fecundity and anovulatory infertility, and women with this disorder are more likely to have gestational diabetes, endometrial cancer, preterm labour, and pre-eclampsia. There are no secular trend data, although the prevalence of PCOS is a comorbidity with increasing rates of obesity (Mason et al., 2008; Burt Solorzano & McCartney, 2010).
- ***Uterine fibroids (also termed leiomyomata) are the most common tumour of the female reproductive tract, possibly affecting up to 25-50% of pre-menopausal women*** (Walker & Stewart, 2005; Baird et al., 2003). They are a significant cause of pelvic pain, abnormal uterine bleeding, menorrhagia, infertility and complications of pregnancy including preterm labour (Rice, Kay & Mahony, 1989; Carlson, Miller & Fowler, 1994a;1994b; Kjerulff et al., 1996; Rowe et al., 1999; Coronado, Marshall & Schwartz, 2000). Fibroids are the leading cause of hysterectomies, accounting for over 200 000 of these surgeries annually in the USA alone (Buttram & Reiter 1981) at an estimated cost of $1.7 billion per year. Risk factors include age, obesity, race, metabolic syndrome and early age at menarche.
- ***Endometriosis occurs in 10-15% of women of reproductive age (15-49) and a minimum of 176 million women worldwide, and in up to 50% of women with infertility and/or chronic pelvic pain*** (Rogers et al., 2009; Adamson, Kennedy & Hummelshoj, 2010)**.** The prevalence of endometriosis is higher in infertile or sub-fertile women than in the general population and has been also been linked to increased risk of endometrial and clear cell ovarian cancer, non-Hodgkin's lymphoma, and atopic disorders (Giudice, 2010). The pelvic pain associated with endometriosis is a major cause of disability and compromised quality of life. Early menarche, short and heavy menstrual cycles, and cycle irregularity are risk factors for endometriosis.

These three disorders, described above, are causes of infertility or sub fertility.

- ***Data from the United States show that the percentage of women who have difficulty in achieving and maintaining pregnancy has increased between 1982 to 2002*** (Swan et al., 1999; NSFG, 2013), ***and is slightly lower in 2006-2010 (though still higher than 1995 and earlier).*** While some of this increase is likely due to people starting families later in life (fertility decreases with age and miscarriage rates increase with age), this does not explain why the sharpest increase in reported infertility is seen in younger women between 1982 and 2002 (Crain et al., 2008).
- ***In the United States, United Kingdom and Scandinavia, the preterm birth rate has increased by more than 30% since 1981*** (Institute of Medicine, 2007; Martin et al., 2009). Since 1990, the percentage of infants born in the USA with low birth weight also rose 16% to 8.1% of births in 2004 (Hamilton et al., 2005). This is of concern because infants born preterm and/or with low birth weight experience significantly higher rates of morbidity and mortality, including respiratory and neurological conditions, during the perinatal period than term and normal birth weight infants. They are also more likely to suffer from cardiovascular disease, obesity, lung disease, and type 2 diabetes in adulthood (Resnik & Creasy, 2004).
- ***There is a secular trend toward earlier onset puberty among American and European girls*** (Euling et al., 2008; Castellino et al., 2005; Semiz et al., 2008). Premature puberty can lead to reduced adult height and is also associated with a higher risk of breast cancer and polycystic ovary syndrome (DiVall & Radovick 2009). It can also have psychological consequences such as greater likelihood of engaging in risky behaviours (smoking, unprotected sex, alcohol and drugs; Cesario & Hughes, 2007).

Genetic and environmental factors (including diet, age, exercise habits, sexually transmitted diseases, and access to good health care) play a role in a woman's overall reproductive health and thus could contribute to these disorders. Moreover, effects of chemicals seen in exposed wildlife and in laboratory animals, similar to those seen in human populations and in DES-exposed individuals, have caused the scientific community to consider whether endocrine disruptors could also cause an increasing variety of reproductive health problems in women, including altered mammary gland development, irregular or longer fertility cycles, and accelerated puberty (Crain et al., 2008; Diamanti-Kandarakis et al., 2009; Woodruff et al., 2008). These changes indicate a higher risk of later health problems such as breast cancer, changes in lactation, or reduced fertility.

Alongside the evidence of female reproductive health diseases and disorders in humans, data describing patterns of reproductive dysfunction in female wildlife have expanded over the past ten years, albeit effects on female reproductive health have been little studied compared with those on the male. In many cases, these patterns appear to mirror those observed in humans, in that the affected wildlife populations appear to exhibit a suite of symptoms that is consistent with exposure to estrogen/anti-androgen and/or androgens. The symptoms recorded often reflect the comparative endocrinology of humans and wildlife, indicating that the human and wildlife evidence for endocrine disruption should perhaps be considered in parallel when assessing whether EDCs contribute to the etiology of female reproductive disorders (for example, see Guillette & Moore, 2006; Edwards, Moore & Guillette, 2006).

There are some well-known and closely-studied examples of female reproductive system disorders in wildlife, which are discussed in the following sections. In many of these wildlife examples, the available evidence supports the involvement of chemicals in the causation of reproductive dysfunction and disease. Evidence includes:

- ***Population declines in Baltic grey seals during the 1950s*** and their more recent recovery (Olsson, Karksson & Ahnland, 1994; O'Hara & Becker, 2003). A high incidence of uterine fibroids (leiomyomata) was found to be correlated with the body burden of organochlorine contaminants in these seals (especially PCBs; Bergman & Olsson, 1986; Bergman, 1999).
- ***Dramatic declines in juvenile recruitment in a population of American alligators*** exposed to chlorinated pesticides, concomitant with abnormal ovarian morphology, large numbers of polyovular follicles and polynuclear oocytes (Guillette & Moore, 2006).
- ***Reproductive endocrine disruption across a range of bird species*** correlated with high concentrations of persistent organic pollutants (e.g. Bosveld & Van Den Berg 2002)
- ***Reduced fecundity, alterations in the timing of sexual maturity and reproduction and premature atresia*** (or degeneration and reabsorption of preovulatory follicles) in some populations of fish in rivers receiving sewage treatment works effluent (reviewed in Tyler & Jobling, 2008).
- ***Masculinisation of female snails*** exposed to the antifoulant tributyltin (TBT), causing blockage of the oviduct resulting in sterility and leading to population declines (Galante-Oliveira et al., 2011; Gibbs & Bryan, 1986; Ellis & Pattisina, 1990).

In addition to wildlife studies, there are data from domestic animal studies that are pertinent here. For example, one of the earliest documented cases of female reproductive dysfunction through exposure to estrogenic compounds concerns breeding problems reported in adult sheep grazing on a type of phytestrogen (isoflavone)-rich clover (Bennetts, Underwood & Shier, 1946). Several biological effects were subsequently associated with reduced fertility in these female sheep, including increased teat length, gestational period and uterine weight, as well as an increased rate of prolapsed vagina, cervix and rectum (Cox & Braden, 1974; Trenkle & Burroughs, 1978). Thus the sheep have provided a useful model for the effects of phytoestrogens. Similarly, it is noteworthy that in some farmed and domesticated animals such as dogs, cats and guinea pigs, as in humans, fibroids are the most common tumour of the genitalia. However, this tumour is normally rare in other species. Human and animal populations may be affected by specific disorders because they share the same habitat (e.g. urban, rural, indoor, and outdoor) and are exposed to similar types of contaminants. Further studies on these domesticated and companion animal species could provide opportunities to learn more about causation and also serve to highlight the similarities between effects occurring in humans and in other vertebrate species.

Hormonal mechanisms underlying female reproductive disorders and diseases

Mechanistic evidence suggests that a proportion of female reproductive endocrine disorders in both humans and wildlife are likely caused by exposures to estrogens, androgen excess or insufficiency, and/or by an imbalance between estrogens and androgens during critical times during the life cycle (e.g. when the ovaries and genitalia are differentiating and/or during puberty when the organs are maturing). Normal hormonal signalling at these times is critical to future reproductive health. For example, at birth the early development of the ovarian follicles depends on the balance between estrogen and other hormones within the developing ovary and, if this is disrupted, ovarian follicle formation and function can be impaired (Dupont et al., 2000). This is believed to lead to ovarian disorders in women and vertebrate wildlife species, like premature ovarian failure (POF). Pre- and/or post-natal exposure to androgens, e.g. in sheep (Hogg, McNeilly & Duncan, 2011), primates (Abbott Tarantal & Dumesic, 2009) and rats (Tyndall et al., 2011) can lead to a polycystic ovary syndrome-like phenotype, including a metabolic component. Importantly, these disorders, both of which can impair fertility, would not be seen until after puberty. It has also been suggested that a changing endocrine environment underlies the age-related increase in human aneuploidy (Hunt & Hassold, 2008). High concentrations of estradiol are required for normal meiotic maturation, although the existence of an endocrine mechanism in the onset of meiosis in the fetal ovary has not yet been explored.

A further example is given by the development of the Müllerian ducts that irreversibly differentiate and proliferate in utero into the oviducts, uterus, cervix and upper vagina (in humans between 9.5 and 11.5 weeks of gestation; Neill, 2006). In vertebrate wildlife species and in laboratory models for human health, differentiation of the external and internal reproductive organs is known to be controlled mainly by the secretion of sex steroid hormones. Abnormal differentiation of the external genitalia has been observed in numerous species

due to exposures to androgens or anti-androgens prenatally (e.g. Jackson, Timmer & Foster, 2008; and c.f. below). As in humans, activation of both the androgen and estrogen receptors (and genes downstream of these) play critical roles in normal female development and the balance of androgen and estrogen may be more important than the action of either of these hormones alone.

Evidence for endocrine disruption of the female reproductive system in humans and wildlife

Apart from the historical case of DES (Chapter 2.1), most of the available evidence concerning the relationship between environmental chemical exposures and female reproductive disorders comes from studies of adults rather than neonates, and often from exposures to persistent organic pollutants (e.g. DDT, PCBs and dioxins), rather than to more modern chemicals (see Chapter 3 for discussion of exposure). The understanding of the contribution of other EDCs beyond legacy persistent and bioaccumulative chemicals has only recently expanded to other chemicals that may influence these outcomes. As previously discussed, it is well established scientifically that environmental exposures during critical periods of growth and development can contribute to an increased risk of future disease or dysfunction later in life (Newbold & Heindel, 2010). In particular, an ovarian dysgenesis syndrome has been proposed which posits that alterations in ovarian structure or function could lead to a syndrome of various gynaecologic disorders, impaired fecundity or later onset adult disease (Buck Louis, Cooney & Peterson, 2011). There is evidence that reproductive dysgenesis in females occurs in response to high environmental exposure levels, such as those encountered occupationally or during chemical accidents or spills (reviewed in Crain et al., 2008). However, evidence is lacking as to whether lower levels of exposure, such as those encountered by the majority of human and wildlife populations, pose a risk to female reproductive development; these studies have not been done.

2.2.2 Evidence for endocrine disruption of the female reproductive system in humans and in mammalian models of humans (rodents and primates)

2.2.2.1 Puberty

Human puberty can be divided into several stages, the first of which involves breast development. This is closely followed by the formation of pubic hair and completes, approximately two years later, with the occurrence of menstruation (menarche).

The average age of menarche has been 13 years of age for the last several decades, whereas some 200 years ago it occurred around 17 years of age (Aksglæde et al., 2008; 2009a). Whilst it is generally accepted that changes in general health and nutrition have most likely caused this advancement (Parent et al., 2003), the more recently reported increased proportion of young girls that develop breasts at aged 7-8 when compared with 10-20 years ago is not so easily explained. Both American (PROS, NHANES III, BCERC) and European studies document this earlier breast development (Biro et al., 2010; Herman-Giddens et al., 1997; Sun et al., 2002; Wu, Mendola & Buck, 2002; Chumlea et al., 2003; Aksglaede et al., 2009b; Semiz et al., 2008; Castellino et al., 2005), as compared to previous studies (e.g. Euling et al., 2008; Reynolds & Wines, 1948; Nicolson & Hanley, 1953).

Up to 86% of the variance in pubertal timing can likely be explained by genetic factors (Parent et al., 2003; Wehkalampi et al., 2008), and a role for increased body mass index and childhood obesity are also indicated by several studies (e.g. Kaplowitz 2001; Bau et al., 2009). The most recent studies, however, suggest that obesity alone cannot explain earlier puberty onset (Aksglaede et al., 2009a) and that other environmental factors are involved (Mouritsen et al., 2010).

Hormonal mechanisms underlying puberty

Pubertal onset is regulated by gonadotropin releasing hormone (GnRH) neurons in the central nervous system. Puberty starts when pulsatile GnRH secretion stimulates the pituitary cells to secrete other hormones (the gonadotropins, follicle stimulating hormone, FSH, and luteinizing hormone, LH) that act on the gonads. The testes and ovaries then start to secrete sex steroid hormones (estrogens and androgens) that induce secondary sexual characteristics such as breast development in females and facial hair in males. Endocrine disruptors could affect puberty through affecting the neuronal circuits and interactions in the brain or directly on the gonads as steroid hormone agonists or antagonists. Furthermore, the same compound can be an agonist when the endogenous hormone level is very low (childhood) and an antagonist when the endogenous hormone level is high (adulthood; see Chapter 2.3).

In recent years, there have been large advances in our understanding of the endocrine control of puberty, particularly in neuroendocrine mechanisms shared with metabolic control, thus providing a mechanistic explanation for the association of obesity with early pubertal onset. The major breakthrough was the discovery of the kisspeptins, hormones that stimulate secretion of GnRH at puberty via interaction with their receptors in GnRH neurons (Navarro et al., 2004). A year later, the regulation of kisspeptin and its receptor by estrogens and androgens was demonstrated in rodent animal models, thus establishing the GnRH-producing neurons as putative key targets of endocrine disruptors affecting the timing of puberty (Navarro et al., 2005; Tena-Sempere, 2010).

Evidence for the role of EDCs in causing early puberty in mammalian models of humans (rodents and primates)

Despite differences in the neuroendocrine control of initiation of puberty, there is convincing evidence from experimental studies with both rodents and primates that prenatal and/or neonatal treatment with estrogen receptor agonists accelerates pubertal onset (GnRH release) in a dose-dependent fashion, whilst the aryl hydrocarbon receptor (AhR) agonists such as

dioxins result in delayed vaginal opening in the female rat (reviewed in Buck Louis et al., 2008). In female rodents, there is evidence for several critical developmental windows that are particularly sensitive to the accelerating influence of estrogenic chemicals on vaginal opening (reviewed in Rasier et al., 2006). These are:

1) The prenatal/postnatal period of reproductive tract development
2) The prenatal period of brain differentiation, and
3) The prepubertal period of brain development.

A few chemicals (exposure to which is described in Chapter 3) have been studied in both animals and humans (e.g. dioxins (TCDD), lead, DDE (metabolite of the pesticide DDT), PCBs and pharmaceutical estrogens), and similar findings tend to be observed across these species. There is recent evidence that neonatal administration of estradiol benzoate or BPA to rodents impairs the release of kisspeptin from the hypothalamus of the brain during pre-puberty, resulting in a dose dependent decrease in luteinizing hormone concentrations in the pituitary (Navarro et al., 2009). This same effect can be seen in offspring from female dams who were undernourished during gestation compared with those that received normal nutrition (Iwasa et al., 2010), thus illustrating a similar effect of chemical exposure and nutrition on puberty in this case.

Epidemiological evidence for EDCs causing early puberty (reviewed in Toppari & Juul, 2010)

Several local epidemics of precocious (early) puberty have been reported (Comas, 1982; Fara et al., 1979). Normal puberty is started by the activation of the whole hypothalamic-pituitary-gonadal axis. In central precocious puberty (CPP) this activation occurs abnormally early. In peripheral precocious puberty, the hypothalamus and the pituitary are not activated, but the hormonal stimulation of pubertal development comes from exogenous agents or autonomously functioning gonads, adrenals or endocrine tumours. Endocrine disruptors with intrinsic sex hormone activity are typical exogenous agents causing precocious puberty. There have not been signs of CPP, but rather of peripheral precocious puberty that has been reversible in many instances. Unfortunately the causes were not identified with sufficient certainty. There are also some regions with a high incidence of CPP, e.g. in Northwest Tuscany (Massart *et al*., 2005), but the possible causes remain speculative (pollution from local greenhouses and several small navy yards).

Children from developing countries who move to industrialized and rich environments have an increased risk of developing CPP (Parent et al., 2003) and endocrine disruptors have been hypothesized to contribute (Krstevska-Konstantinova et al., 2001). Twenty six immigrant girls with CPP had relatively high levels of the organochlorine contaminant p,p'-DDE, whereas only 2 of 15 native patients in Belgium had detectable serum DDE concentrations (Krstevska-Konstantinova *et al*., 2001). Early and temporary exposure to weakly estrogenic DDT might stimulate both hypothalamus and pituitary maturation at the same time as it has a direct negative feedback effect on the pituitary gonadotropin secretion, preventing sexual maturation. After moving to a new environment where DDT is no longer used, the decrease in exposure may allow the onset of puberty (Rasier et al., 2006). DDT has a long half life, however, making a sudden decline in internal exposure unlikely. Nevertheless, DDT affects GnRH activity in experimental settings and it is likely that other chemicals with similar mechanisms of action will also (Rasier et al., 2006).

There are many case reports of peripheral precocious puberty in children exposed to pharmaceutical drugs or ointments or food containing sex steroids (Henley et al., 2007). Estrogens stimulate breast development, while androgens induce growth of pubic hair and changes in skin (oily skin and hair, adult-type sweat odour). If the exposure can be stopped, peripheral puberty does not advance and pubertal signs can disappear slowly. Peripheral puberty can also induce central puberty although association of endocrine disruptors with the onset of CPP is less well documented than with peripheral puberty. A summary of the epidemiological studies investigating a role for endocrine disruptors in causing early puberty are summarized in **Table 2.2.**

In summary, there is consistent evidence that exposure to lead is associated with a slight delay in puberty, whereas all other exposures studied so far do not show any clear association with the timing of puberty except for polybrominated biphenyls that were linked to an early age at menarche and pubic hair development. Taking all of the evidence together, whilst there is biological plausability that exposure to endocrine disruptors could contribute to changes in pubertal onset, demonstrated epidemiological associations are absent and warrant further investigation. One of the difficulties concerns the complexity of relating this endpoint with exposures that may have occurred at different times during development and for different durations. Exposure to mixtures have not been considered. There are also many other factors known to influence timing of puberty (e.g. nutrition) that may vary between individuals and populations.

2.2.2.2 Low fecundity, sub fertility, infertility, adverse pregnancy outcomes

Between 3.5 and 16.7% of couples in developed countries and 6.9 to 9.3% of couples in less developed countries experience an inability to conceive (Boivin et al., 2007). Paternal exposures to chemicals resulting in reduced semen quality could have an effect on fecundity (capacity to conceive) as well as specifically on male fertility, which is discussed in the next section. Here we focus on female fecundity and fertility (ability to deliver a live born infant). Sub fertility/infertility can carry increased risks of adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, low birth weight, and fetal death. Causes of low female fecundity and of sub fertility/infertility include ovulatory disturbances, premature ovarian

Table 2.2. Overview of epidemiological studies investigating the effects of endocrine disruptors on onset of human puberty. Adapted from WHO (2012).

Contaminant	Sex	Observation	References
Chlorinated pesticides (DDT and DDE)	Male	No association with pubertal development	Gladen, Ragan & Rogan, 2000
	Female	Younger age at menarche	Vasiliu, Muttineni & Karmans, 2004
		Precocious puberty	Krstevska-Konstantinova et al., 2001
		No association with breast stage or pubic hair development	Wolff et al., 2008
		No association with pubertal development	Gladen, Ragan & Rogan, 2000
Dioxins	Male	No association with sexual maturation	Den Hond et al., 2002
	Female	Later onset of breast development	Leijs et al., 2008
		No association with the onset of menarche	Warner et al., 2004
		Lower stage of breast development	Den Hond et al., 2002
Polychlorinated biphenyls (PCBs)	Female	Slowed breast development	Staessen et al., 2001
		No association with menarche or pubertal stages	Den Hond et al., 2002; Vasiliu, Muttineni & Karmans, 2004
		No association with breast stage or pubic hair development	Wolff et al., 2008
		No association with pubertal development	Gladen, Ragan & Rogan, 2000
	Male	Late first ejaculation	Leijs et al., 2008
		Reduced penile length	Guo et al., 2004
		Slowed genital development	Den Hond et al., 2002; Staessen et al., 2001
		No association with the development of puberty	Mol et al., 2002
		No association with pubertal development	Gladen, Ragan & Rogan, 2000
Polybrominated biphenyls (PBBs)	Female	Earlier age at menarche and pubic hair development	Blanck et al., 2000
Bisphenol A	Female	No association with breast stage or pubic hair development	WHO, 2011
Lead	Female	Delayed breast and pubic hair development	Selevan et al., 2003
		Inversely associated with inhibin B levels	Gollenberg et al., 2010
		Delayed breast development, pubic hair growth and age of attainment of menarche	Naicker et al., 2010
	Male	Delayed onset of puberty on the basis of testicular volume of > 3 mL, genitalia staging and pubic hair staging	Williams et al., 2010
Cadmium	Female	High levels of both cadmium and lead is inversely associated with inhibin B levels	Gollenberg et al., 2010

insufficiency, implantation disorders, aneuploidy and uterine abnormalities such as fibroids. It is important to note here that exposure of the male parent to chemicals can also cause sub fertility, albeit manifest in the female (Silbergeld & Patrick, 2005).

Premature birth rates and low birth weight

Preterm birth is the single largest factor worldwide in infant mortality and morbidity, and the frequency of preterm birth has seen a dramatic rise in developed countries over the last two decades. In the United States, the preterm birth rate has increased more than 30% since 1981, and 8% since 1990 (Institute of Medicine, 2007; Martin et al., 2009; **Figure 2.1**). Most of the increase has been in moderately preterm births (32-36 weeks), though the very early preterm birth rate (less than 32 weeks) has also risen in recent years. Since 1990, the percentage of infants born with low birth weight in the United States has also risen 16% to reach 8.1% of births in 2004 (Hamilton et al., 2005). Low birth weight is defined as birth weight less than or equal to 2500g and encompasses pre term infants and those born at term but whose growth was restricted in utero (intrauterine growth restriction, IUGR), as well as those who are both growth retarded and premature (small for gestational age; GA). These increases in low birth weight and preterm delivery in the USA cannot be explained by increases in multiple births or in vitro fertilisation, changes in medical practice, or other demographic factors (Davidoff et al., 2006; Donahue et al., 2010). Further, there is a persistent racial disparity in adverse birth outcomes, with African Americans having higher rates of low birth weight and preterm delivery (Hamilton et al., 2005; Institute of Medicine, 2007).

Trend data indicate that preterm delivery rates are also increasing in the United Kingdom and in Scandinavian countries (Morken et al., 2008; Beck et al., 2010), and as

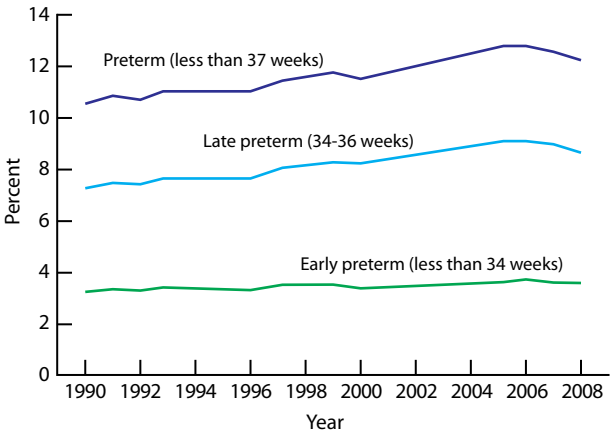

Figure 2.1. Preterm birth rates: United States, final 1990-2006 and preliminary 2007 and 2008. Source CDC/NCHS National Statistics System.

with the USA, are not necessarily explained by changing demographic or medical/delivery characteristics, although social inequalities such as rented and crowded homes, smoking, alcohol consumption and intake of saturated fatty acids are reported to be predictive of preterm delivery in some populations (Morken et al., 2008; Niedhammer et al., 2011).

Birth weight and gestational age at delivery are important predictors of neonatal and infant health. Infants born preterm and/or with low birth weight also experience significantly higher rates of morbidity and mortality during the perinatal and neonatal periods than term and normal birth weight infants, including respiratory and neurological conditions. Low birth weight infants experience longer hospital stays at birth and a greatly increased risk of respiratory distress syndrome. IUGR has been identified as a significant risk factor for chronic hypertension, cardiovascular disease, obesity, lung disease, and type 2 diabetes in adulthood (Resnik & Creasy, 2004). Premature deliveries and low birth weight pose significant challenges to the children born from these pregnancies and women during these pregnancies, and also have a major financial impact on health care systems. For example, average hospital charges for premature births in 2003 in the USA have been estimated to be $18.1 billion, about half the total infant hospital charges for all USA births (March of Dimes, 2006).

Hormonal mechanisms underlying fecundity and fertility

Achieving pregnancy requires a normally functioning hypothalamic-pituitary-ovarian axis, a normal female reproductive tract, normal endocrine homeostasis, and normal semen parameters. The structure and function of the oviduct relies heavily on the coordinated regulation and interaction of progesterone and estrogens (Hess, Nayak & Giudice, 2006). The same hormones also prepare the endometrium for implantation through an orderly process that enables attachment of the embryo to the endometrial epithelium, passage through the epithelium, and invasion of the trophoblast into the maternal decidual compartment (Hess, Nayak & Giudice, 2006). Dysfunction of the Müllerian tissues (oviduct, uterus, cervix and upper vagina) can occur as a result of disturbances in hormonal action and/or production during the preparation of the uterus for pregnancy (Crain et al., 2008). This can result in miscarriage as a result of disturbances in implantation. If the fetus survives beyond the first trimester, then there can be difficulties in pregnancy, such as pre-eclampsia, which can contribute to adverse pregnancy outcomes such as preterm delivery and intrauterine growth restriction. Even a partial withdrawal of progesterone, for example, in late pregnancy can result in reduced fetal growth (Bowman, Streck & Chapin, 2010).

The female menstrual cycle is highly regulated by a variety of hormones. According to Small et al., (2006), 84% of cycle variability is due to variation in the length of the follicular phase. In ageing women, changes in hormonal levels, including increased follicular phase estradiol, results in decreased cycle length and decreased fertility whilst decreased follicular phase estrogen results in lower fecundity (Small et al. 2006). As with puberty, kisspeptins have now emerged as major triggers for ovulation and are also involved in the metabolic control of reproductive function in overweight and underweight women (Roa et al., 2008). Hormone disruptors could interfere with menstrual cyclicity through multiple pathways, resulting in irregular periods, shorter or longer cycles and changes in duration of bleeding and/or pain (Mendola, Messer & Rapazzo, 2008; Mendola & Buck Louis, 2010).

Evidence for a role for EDCs in causing lowered fecundity and/or fertility in mammalian models of humans (laboratory rodents)

Hormonal balance, a proper level of sex hormones, is important to preserve female reproduction and regular estrous cycles, and to maintain fertility in rodent models, as with other vertebrates including humans. This balance can be disturbed by changing concentrations of estrogen, androgen or progesterone or by altering the expression of steroid hormone receptors (e.g. Ortega, Salvetti & Padmanabhan, 2009). Several studies have shown that circulating estradiol concentrations in rodents can be decreased by exposure to several pesticides, including heptachlor, lindane, atrazine, simazine or hexachlorobenzene (see Chapter 3 for a review of exposures to these chemicals). Progesterone concentrations also can be decreased by exposure to methoxychlor, especially during the estrous phase of the estrous cycle in rats. It should be noted, however, that the estrous cycle in non-primate mammals only partly corresponds with the ovarian cycle in humans; estrus is the period of greatest female sexual responsiveness, usually coinciding with ovulation; whereas, diestrus is the luteal phase of the estrous cycle when the female is not receptive to the male.

Organochlorine compounds, atrazine and simazine are all known to interrupt the estrous cycle in rodents through altering hormone synthesis or action. Some pesticides also decrease the number of healthy follicles and increase the number of atretic follicles, potentially reducing fertility. Adverse effects

of endocrine disruptors on the female reproductive system are summarized in Chapter 2.1, **Table 2.1.** Some studies suggest that in utero exposure to endocrine disruptors may alter the mouse estrous cycle, and prematurely end cyclicity altogether (Rubin et al., 2001; Markey et al., 2003; Nikaido et al., 2004; Jefferson, Padilla-Banks & Newbold, 2005).

Several EDCs have been found to cause urogenital dysmorphogenesis in mammalian laboratory models, leading to infertility/sub fertility. TCDD and chlordecone inhibit events responsible for the regression of the Wolffian ducts, which then causes interference with development of the genitourinary system (Silbergeld & Patrick, 2005). Neonatal exposure to BPA, or DES, but not phytoestrogen isoflavones, has been shown to alter Hoxa-10 and Hoxa-11 uterine expression in the rat and thus impair the response of the endometrium to steroid treatment (Varayoud et al., 2008).

Follicles containing two or more oocytes have been observed in wildlife and in animal models in lab studies following prenatal exposure to estrogenic substances, indicating that EDCs can interfere with follicle formation (Crain et al., 2008) and suggesting that this effect might precede the formation of primordial follicles. The transition from primordial follicle to primary follicle is inhibited by estrogen and EDCs such as methoxychlor can inhibit folliculogenesis (Uzumcu et al., 2006).

Evidence for EDCs affecting menstrual cyclicity in women

A review of the results from epidemiology studies evaluating adult environmental chemical exposures and menstrual cycle effects published by Mendola, Messer & Rapazzo (2008) states that there are a number of published studies, but they cover differing types of exposures, and reported effects are contradictory (e.g. longer versus shorter menstrual cycles for different chemicals). This is perhaps not surprising considering that exposures are never to one chemical, but rather to a large number of additional chemicals that may also contribute to the effect in question (Chapter 1.3.8 for a discussion of cocktail effects). Thus, it is difficult to assess the overall effect of environmental chemical exposures on menstrual cycles, given the current limited data, although individual chemicals have been associated with particular effects on menstrual cycles (Mendola, Messer & Rapazzo, 2008). For example, shorter menstrual cycles have been observed among lead-battery plant workers (Tang & Zhu 2003), in women exposed to chlorodibromomethane in drinking water (Windham et al., 2003), or those exposed to DDT (Ouyang et al., 2005; IPCS, 2011). In contrast, longer menstrual cycles have been observed in association with exposure to dioxins (Eskenazi et al., 2007) and hormonally-active pesticides (Farr et al., 2004), elevated serum PCBs (Cooper et al., 2005), or working in the semiconductor industry (Hsieh et al., 2005). Many of these studies also observed associations with other menstrual disorders such as abnormal and/or painful bleeding, and missed periods (Tang & Zhu, 2003; Farr et al., 2004; Cooper et al., 2005). Some studies, however, find no relationship between menstrual abnormalities, cycle length, or other menstrual characteristics and PCB, metals, DDE, or DDT exposure (Yu et al., 2000; Chen et al., 2005). In studies with biomarker data, women exposed to pentachlorophenol had lower follicle-stimulating hormone levels (Gerhard et al., 1999a). Women exposed to DDT and DDE had reduced progesterone and estrogens, but PCBs were not associated with changes in hormone profiles (Windham et al., 2005; Perry et al., 2006).

Epidemiological evidence for EDCs causing lowered fecundity or fertility

Epidemiological evidence of effects of EDCs on female fecundity has been the subject of three recent reviews (Buck Louis et al., 2008; Mendola, Messer & Rapazzo, 2008; Woodruff & Walker, 2008). These studies have generally been restricted to occupational and accidental exposures, mainly to metals and pesticides, as well as to perfluorinated compounds (Fei et al., 2009). Effects include those on menstrual cycles (mirrored by effects on fecundity) and on time to pregnancy (which can be confounded). Interestingly, effects often only become significant in population subsets with other known risk factors such as age or smoking, suggesting that interacting (perhaps additive) effects are occurring. Moreover, opposite effects on female fecundity of prenatal exposure to DDT appear to depend on whether high concentrations of DDT or its metabolite DDE were measured in maternal serum (IPCS, 2011; Law et al., 2005; Harley et al., 2008; Cohn et al., 2003; Gerhard et al., 1999b). This raises interesting questions regarding maternal differences in metabolism and genetic susceptibility to endocrine disruption.

Epidemiological evidence for EDCs causing adverse pregnancy outcomes including pre term delivery

Exposure to endocrine disruptors and other chemicals have also been associated with a variety of adverse pregnancy outcomes, including miscarriage, preeclampsia (characterized by hypertension during pregnancy), IUGR, poor weight gain during fetal development, and preterm delivery (Stillerman et al., 2008; Slama & Cordier, 2010). Sufficient evidence exists that prenatal exposure to lead and glycol ethers can increase the risk of miscarriage (Slama & Cordier, 2010). There is limited evidence that other metals (e.g. mercury and cadmium), chlorinated chemicals (e.g. DDT/DDE) and solvents can increase the risk of miscarriage (Slama & Cordier, 2010; IPCS, 2011). For fetal growth, most evidence is limited, including for metals, PCBs and DDT/DDE, although there is stronger evidence that exposure to perfluorinated chemicals can affect fetal growth outcomes (Slama & Cordier, 2010).

Limited evidence exists that exposures to metals or organochlorine and organophosphate pesticides can increase the risk of preterm delivery (Slama & Cordier, 2010). There are several studies evaluating the relationship between phthalates and gestational length. However, studies reporting both increased or decreased gestational length have been found, and the current epidemiologic evidence is insufficient to draw a conclusion about the mechanisms underlying such a relationship (Slama & Cordier, 2010). Similarly, it has been hypothesised that BPA can adversely affect pregnancy outcomes

(Ranjit, Siefert & Padmanabhan, 2010), and a small nested case control study from Mexico City found an association between BPA and preterm delivery (Cantonwine et al., 2010). Conclusions based on this single small study are not possible; more studies are needed.

The importance of steroidal hormones in implantation means that adverse pregnancy outcomes are a plausible consequence of endocrine disruption, albeit they are difficult to study because of the possibility that trends may be obscured by confounders for maternal age and weight and by the quality of the prenatal care available. Over the last 10 years, there have been advances in our understanding of the endocrinology underlying implantation, however, and there are now suggestions that chemical exposures could influence birth outcomes other than urogenital abnormalities.

Menopause

There are a few studies of the relationship between chemical exposure and effects on menopause. Three studies of PCB exposures have found no relationship with changes in age at menopause (Yu et al., 2000; Blanck et al., 2004; Cooper et al., 2005). However, some studies have found a relationship between DDT/DDE or dioxin exposures and early age at menopause (Cooper et al., 2002; Akkina et al., 2004; Eskenazi et al., 2005; IPCS, 2011). In contrast, DDT exposure in the Agricultural Health Study was associated with slightly older age at menopause (Farr et al., 2006). Most of these studies are cross-sectional, which could be insufficient to detect relevant exposures if they occur earlier or during critical periods of development.

2.2.2.3 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a disorder affecting both metabolism and reproduction and occurs in 3 to 15% of women of reproductive age, depending on the population studied and the diagnostic criteria applied (Teede, Deeks & Moran, 2010; Broekmans et al., 2006). It has had multiple diagnostic criteria, although it is typically characterized by hyperandrogenism (abnormally high circulating testosterone concentrations), oligo/amenorrhea (irregular or absent periods or abnormal bleeding), and polycystic ovaries (i.e. those with an overabundance of maturing follicles) (Mendola & Buck Louis, 2010). A recent definition of PCOS requires two of the following three criteria: (1) clinical or biochemical evidence of hyperandrogenism; (2) intermittent or absent menstrual cycles; and (3) polycystic ovary morphology as visualized by ultrasound (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). PCOS is the leading cause of sub-fecundity and anovulatory infertility, and women with this disorder are more likely to have gestational diabetes, preterm labour, and preeclampsia, endometrial cancer and infertility (Azziz et al., 2004); up to 10% of women with this disorder develop diabetes during the third or fourth decade (Norman et al., 2007). Many body systems are affected resulting in ovulatory dysfunction, infertility, obesity, acne and metabolic syndrome, all of which have personal, social, and economic consequences. The prevalence of PCOS has risen with increasing rates of obesity (Mason et al., 2008; Burt Solorzano & McCartney, 2010).

Impaired fetal growth as well as a higher weight at birth have been associated with the subsequent development of PCOS during adulthood. It is also associated with early puberty, although associations with age at menarche are unclear (Hart, Hickey & Franks, 2004).

Hormonal mechanisms underlying PCOS.

Various mechanisms underlying this disorder have been proposed, although the origins and etiology remain largely unknown (Mendola & Buck Louis, 2010; Goodarzi et al., 2011). Hypothesised etiologies include in utero environmental exposures leading to fetal reprogramming during critical windows of follicle formation and follicle activation (Mendola & Buck Louis, 2010), primary ovarian abnormality, or deregulation of fat metabolism (Mendola & Buck Louis, 2010). The latter has received the least attention, albeit obesity–related hyperinsulinemia may lead to hyperandrogenism during puberty and promote progression towards a PCOS phenotype. Evidence for a fetal origin for the development of PCOS phenotypes stems from animal models and epidemiological studies. For example, excessive prenatal testosterone (T) exposure is hypothesized to be a mechanism underlying PCOS (Dumesic, Abbot & Padmanabhan, 2007), with evidence from experimental animal studies of rhesus monkeys, rats and sheep (West et al., 2001; Abbott et al., 2005; Forsdike et al., 2007) showing that exposure of fetuses to testosterone when target organ systems are differentiating alters their ontogenic development and phenotypic expression such that they mimic characteristics of the phenotypes characteristic of PCOS in women. The full spectrum of symptoms associated with PCOS is not apparent until puberty and thus a "two hit" hypothesis has also been proposed in which prenatal testosterone exposure leads to hyperandrogenism which then impairs the sensitivity of the brain to feedback mechanisms such that sustained hyperandrogenism is maintained, eventually leading to PCOS (Bremer, 2010). Yet another mechanism of fetal programming by androgen excess is epigenetic changes in gene expression, recently demonstrated in a PCOS mouse model (Zhu et al., 2010). This may explain the heterogeneous phenotypic expression of PCOS that occurs in sisters with the same genotype (Diamanti-Kandarakis et al., 2006).

Epidemiological and animal studies linking EDCs exposure to PCOS

There is definitely a genetic basis for PCOS but the heterogeneity of its features even within families suggests that the gestational environment and lifestyle are of prime importance (Norman et al., 2007). Few studies have been carried out to evaluate the relationship between PCOS and exposure to EDCs. One EDC that has been associated with PCOS, however, is BPA where two studies of adults found a relationship between serum BPA levels and women with PCOS

(Takeuchi et al., 2004; Kandaraki et al., 2011). It is possible that the elevated BPA is a consequence, and not a cause, of PCOS as women with PCOS have higher circulating testosterone levels than healthy women and these elevated androgen concentrations decrease BPA clearance (Takeuchi et al., 2006). However, at least one animal study supports the case for BPA exposure playing a role in PCOS in rodents (Fernandez et al., 2010).

Overall, genetic markers for PCOS are still not established and its etiology is not known, albeit it seems consistent with an interaction between genetic susceptibility and environmental factors, including lifestyle. Given the hormonal basis for this disease, a role for EDCs as risk factors should be considered. Prospective epidemiological studies have not been conducted and chemical test methodologies have not been devised.

2.2.2.4 Uterine fibroids

Uterine fibroids (leiomyomata), benign tumours that arise from the uterine myometrium, are the most common tumour of the female reproductive tract (Walker & Stewart, 2005). They are a significant cause of pelvic pain, abnormal uterine bleeding, infertility and complications of pregnancy including preterm birth (Rice, Kay & Mahony, 1989; Carlson, Miller & Fowler, 1994a; 1994b; Kjerulff et al., 1996; Rowe et al., 1999; Coronado, Marshall & Schwartz, 2000). Uterine leiomyomatas occur in 25-50% of all women in countries in which this has been studied, although the prevalence estimates are mostly based on clinical cases, and may not, therefore, reflect the true incidence (Baird et al., 2003). A study in the USA of randomly selected women from the northeast, independent of clinical symptoms, found that cumulative incidence of fibroids by age 50 was almost 70% for Caucasian women and greater than 80% for African-American women (Baird et al., 2003). One other published study has reported prevalence of fibroid tumours using transvaginal ultrasound screening from a random sample of women from Sweden (Borgfeldt & Andolf, 2000). Prevalence was much lower, with 5% of women aged 25 to 40 and 8% of women 33 to 40 years old having fibroids. Prevalence for a comparable age range in the Baird et al. study is 26% for white women and 53% for black women.

Time trend data are not available on fibroid incidence. However, they are the leading cause of hysterectomies, accounting for over 200 000 of these surgeries annually in the USA alone (Buttram & Reiter, 1981) at an estimated cost of $1.7 billion per year. The rates of hysterectomies in the United States have declined slightly, although they have increased for certain reproductive conditions (e.g. bleeding and pain) (Farquhar & Steiner, 2002). While these tumours rarely metastasize, they can have a significant and negative impact on a woman's health (Rice, Kay & Mahony, 1989). This is because even in fertile women, fibroids have been implicated in recurrent pregnancy loss and first and second trimester miscarriage. In late pregnancy, fibroids can enlarge and cause obstetric complications.

Hormonal and anatomical changes associated with menstruation and pregnancy influence uterine fibroid incidence, as does obesity and metabolic syndrome. Earlier and later age at menarche have been associated with increased and decreased risk, respectively (Crain et al., 2008; Okolo 2008; Terry et al., 2010). The risk of fibroids also increases with age during premenopausal years but thereafter disappears.

Hormonal mechanism underlying fibroids

Fibroids have significantly higher concentrations of estrogen receptors compared with normal uterine tissue, despite the fact that circulating levels of steroid hormones in women with clinically detectable fibroids are no different from those measured in normal women (Blake, 2007; Okolo, 2008). In in vitro cultures, rodent fibroid cells proliferate in response to estrogen and this response is inhibited by estrogen antagonists (Othman & Al-Hendy, 2008). There is also a role for progesterone in promoting fibroid growth and recent literature indicates that fibroid growth is stimulated in response to a mixture of progesterone and estrogens, rather than one or the other (Ishikawa et al., 2010).

Laboratory evidence for EDCs causing fibroids in rodent models of humans

A small collection of studies in laboratory rats and/or mice has demonstrated that exposure to some EDCs can increase the incidence of uterine fibroids (reviewed in Crain et al., 2008). These effects reported in rats and mice inform human epidemiology studies and add credence to the hypothesis that fibroids in humans can be induced by EDC exposure (McLachlan, Newbold & Bullock, 1980).

In addition to these in vivo studies, in vitro studies also suggest that EDCs contribute to the growth of uterine fibroids. Rat uterine leiomyoma cells are extremely sensitive to estrogenic EDCs, with physiologically relevant concentrations of kepone, α-endosulfan, or 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE, a breakdown product of the pesticide methoxychlor) stimulating cell proliferation, and HPTE, methoxychlor, kepone, α-endosulfan, β-endosulfan, toxaphene, or dieldrin increasing transcription in an estrogen-sensitive reporter gene assay (Hodges et al., 2000; for a review of human exposure to these chemicals, see Chapter 3). DES also induces proliferation of rat uterine leiomyoma cells, indicating that such cells are sensitive to estrogenic pesticides and pharmaceutical agents (Hodges et al., 2001).

Although evidence for a fetal origin of uterine fibroids in humans is controversial, in rodent models (CD-1 mice and Eker rats), fetal exposures to both DES and bisphenol A during particular periods in development have been shown to cause increased risk of fibroids in adulthood (Newbold, Jefferson & Padilla-Banks, 2007; Crain et al., 2008).

Epidemiological evidence for EDCs causing fibroids

There are scant data from humans evaluating EDCs and fibroids, and these studies have mostly focused on adult exposures (Mendola, Messer & Rapazzo, 2008). One study demonstrated that women exposed to high levels of TCDD from an industrial accident were less likely to have fibroids,

leading the authors to speculate that this was because TCDD may have an anti-estrogenic effect (Eskenazi et al., 2007). Another study of women who were sport fishers or sport fish consumers from the Great Lakes area and potentially exposed to higher organic pollutants including DDE and PCBs found that while the activity itself was associated with a modest increased risk of fibroids, circulating levels of the chemicals themselves were not associated (with the exception of PCBs in a subpopulation) with higher disease outcomes (Lambertino et al., 2011). Finally, two studies of women exposed to phthalates found an association with fibroids. In a relative small study of women in Taiwan, researchers found higher levels of the metabolites of the phthalate plasticizers diethylhexylphthalate (DEHP) and dibutylphthalate (DBP) in women with fibroids compared to controls, and a higher risk for women who were glutathione S-transferases M1 null, suggesting genetic susceptibility to phthalate effects (Huang et al., 2010). A larger, cross-sectional study of USA women also found an association with uterine fibroids and the metabolite of DBP (Weuve et al., 2010). Human exposure to phthalates is discussed in Chapter 3.

2.2.2.5 Endometriosis

Endometriosis is a major cause of infertility and chronic pelvic pain in women, and has also been linked to increased risk of endometrioid and clear cell ovarian cancer, non-Hodgkin lymphoma, and atopic disorders (Giudice, 2010). Endometriosis is characterized by the presence and growth of endometrial glands and stroma outside the uterus, primarily in the pelvic and abdominal cavities, but can also rarely be found in the thoracic cavity and the brain (Giudice, 2010). The growth produces hemorrhages or vesicles appearing as brown, black or blue lesions (Mendola & Buck Louis, 2010). Pelvic pain associated with endometriosis is a major cause of disability and compromised quality of life. Estimates for the incidence of endometriosis vary, often by study population. While most studies find that between 10% and 15% of reproductive-age women have endometriosis (Leibson et al., 2004; Vigano et al., 2004), this could be influenced by difficulties in diagnosis. A study of USA women in 2007-09 found that while MRI-visualized incidence was 11%, 34% of women had endometriosis as diagnosed by surgical, MRI and histological confirmation (Buck Louis et al., 2011), indicating that the true incidence among the population could have been previously underestimated. Incidence is much higher (between 35% and 50%) in women with pelvic pain, infertility, or both (Cramer & Missmer 2002). In the USA in 2009, endometriosis was estimated to cost $69 billion in health care and loss in productivity (Hummelshoj & O'Hooghe, 2012).

Early menarche, short and heavy menstrual cycles, and cycle irregularity have fairly consistently been associated with an increased risk of endometriosis (Vigano et al., 2004; Matalliotakis et al., 2008; McLeod & Retzloff, 2010) and late age at menarche has been suggested to be protective (Treloar et al., 2010). Family history, genetic predisposition, race and social status have all been explored as possible factors in the etiology of endometriosis with conflicting results (Falconer, D'Hooghe & Fried 2007; Vigano et al., 2007; Montgomery et al., 2008; Guo 2009; Siristatidis 2009). The most recent study (Guo, 2009) argues that genetic polymorphisms contribute little to disease risk, and that endometrios may be largely an epigenetic disease (for a discussion of epigenetics see Chapter 1.3.6).

Hormonal mechanism underlying endometriosis

Endometriosis is at least partially dependent on estrogen. Uterine endometrial gland development (adenogenesis) in humans begins in utero and is completed during puberty (Yin & Ma, 2005). Adenogenesis is influenced by growth factors, tissue remodeling factors, and steroid hormones that alter cell proliferation and extracellular matrix remodelling (Gray et al., 2001). All of these can thus be affected by altered hormonal signalling during early development, which can, in turn, adversely affect adult uterine morphology and function. One condition that could result, albeit not until adult life, is endometriosis. An in utero origin for this disorder has, however, only recently been proposed; until relatively recently, the primary hypothesis for endometriosis was that it was initiated by retrograde menstruation (backward movement of menstrual fluids through the fallopian tubes and into the peritoneal cavity; Sampson 1927). However, as retrograde menstruation occurs in the majority of women (Halme et al., 1984) and fewer women develop endometriosis, other initiation pathways have since been explored and it is now hypothesised that influences on in utero development can contribute to future endometriosis risk (Messmer, 2004; Bulun, 2009; Mendola & Buck Louis, 2010). Moreover, the immune system is also thought to play a role (see Chapter 2.11; Hompes & Mijatovic, 2007; Crain et al., 2008; Giudice, 2010; Mendola & Buck Louis, 2010). In either case, however, it appears that estrogen is necessary for progression of endometriosis (Dizerega, Barber & Hodgen, 1980), and aromatase in endometriosis lesions converts androgens to the estrogen estrone (E1) which is then converted to estradiol (E2) by other steroidogenic enzymes. Prostaglandin E2 is also a potent inducer of aromatase activity (Bulun, 2009).

Evidence for EDCs causing endometriosis in mammalian models of humans (rodents and primates)

Endometriosis occurs spontaneously only in primates, as estrous animals do not shed their endometrial tissue and do not develop endometriosis. Several studies in primates have found a relationship between adult exposures to dioxins and endometriosis. This includes a study of twenty rhesus monkeys dosed and followed for 15 years, which reported an increase in incidence and severity with higher dioxin exposures (Rier et al., 1993; Rier et al., 2001). Another study in the cynomolgus monkey found implants of endometrial tissue in the pelvic cavity survived longer and grew larger in animals exposed for one year to high doses (17.86 ng/kg per day) of TCDD (Yang, Agarwall & Foster, 2000).

Rodent models (mainly rats and mice) of endometriosis have been developed, where the disease is induced by

surgical transplantation or endometrial tissue into ectopic sites. Studies with these models find that endometriosis can be promoted by many organochlorines, including the dioxin TCDD, the pesticides methoxychlor and DDT, or many PCBs with dioxin-like effects (Birnbaum & Cummings, 2002). In several studies, fetal exposures also have been found to promote future endometriosis. For example, mice exposed to TCDD on gestational day 8 had increased size of implanted endometriotic lesions when combined with an adult exposure (Cummings, Hedge & Birnbaum, 1999). There is increasing evidence that epigenetic changes (see Chapter 1.2.5) are involved in endometriosis and there is convincing evidence that such changes can be induced by in utero exposure to exogenous chemicals (Guo 2009; Cakmak & Taylor, 2010). A recently published study showed that dioxin exposure of mice during fetal development led to a progesterone resistant phenotype that persisted for several generations (Bruner-Tran, Ding & Osteen, 2010).

Epidemiological evidence for endocrine disruptors causing endometriosis

Most research on the role of environmental exposures has focused on adults. While some studies find that dioxins are associated with endometriosis, several studies find no relationship (Mayani et al., 1997; Pauwels et al., 2001; Eskenazi et al., 2002; Heilier et al., 2005; Porpora et al., 2009; Rozati et al., 2009; Simsa et al., 2010). Reviews point out that often these epidemiologic studies are limited to specific or uniquely exposed populations (e.g. infertile women) (Mendola, Messer & Rapazzo, 2008). Most studies have found a relationship between PCB exposures and increased risk of endometriosis, or higher levels of serum PCBs among cases when compared to controls (Gerhard & Runnebaum, 1992; Mayani et al., 1997; Birnbaum & Cummings, 2002; Heilier et al., 2004; Buck Louis et al., 2005; Porpora et al., 2006). Several studies have also found a relationship between circulating phthalate (and phthalate esters) and endometriosis (Lebel et al., 1998; Pauwels et al., 2001; Buck Louis et al., 2005; Porpora et al., 2006; Hoffman et al., 2007; Porpora et al., 2009; Rozati et al., 2009; Cooney et al., 2010; Trabert et al., 2010), although the associated phthalate ester varies among the study (primarily metabolites of DEHP and DBP) (Cobellis et al., 2003; Reddy et al., 2006; Huang et al., 2010; Weuve et al., 2010). One cross-sectional study of USA women found a relationship between endometriosis and cadmium (Jackson, Zullo & Goldberg, 2008). Human exposure to these chemicals, particularly phthalates, can be significant (for a review of human exposures, including in breast milk, see Chapter 3).

There are fewer data on human populations examining the relationship between endometriosis in adulthood and EDC exposures during the fetal or early life period. However, a large, prospective cohort study found that daughters of women who took DES during pregnancy had an increased risk of endometriosis (Missmer et al., 2004), suggesting that exposure to EDCs during fetal development can increase risk of future endometriosis. This is biologically plausible because the development of the uterus and endometrium from the uterine mesenchyme depends on specific developmental pathways involving estrogen receptor alpha (ER-alpha) and specific transcription factors, such as HoxA10, bone morphogenetic proteins (BMPs), and leukemia inhibitory factor (LIF) (Bulun, 2009).

There is a strong genetic component in endometriosis in some patients and there may be specific genotypes that are more susceptible to developing the disorder (Painter et al., 2010). This is an area of great importance for future research on fetal, neonatal/childhood, adolescent, and adult exposures to endocrine disrupting chemicals.

2.2.3 Evidence for endocrine disruption of the female reproductive system in wildlife

2.2.3.1 Wild mammals

There are several correlative studies indicating that EDCs could have an impact on reproductive hormones in both seals and polar bears (Haave et al., 2003; Oskam et al., 2003; Oskam et al., 2004). High concentrations of hydrophobic contaminants (particularly PCBs, organochlorine pesticides and brominated flame retardants) have been measured in the marine mammals which, with the exception of plankton-feeding whales, are top predators (Ross et al., 2000; Aguilar, Borell & Reijnders, 2002; Hansen et al., 2004; Lie et al., 2004; Noel et al., 2009; see also Chapter 3.2.1). In Baltic grey seals, for example, population declines during the 1950s were related to exposure to these chemicals (Olsson, Karlsson & Ahnland, 1994). Critical to this conclusion was the fact that the high incidence of uterine fibroids (up to 65% of females 22–41 years old) was found to be positively correlated with the body burden of organochlorine contaminants (especially PCBs; Bergman & Olsson, 1986; Bergman, 1999). Moreover a 60% decrease in the number of females becoming pregnant was observed among ringed seals from PCB contaminated Bothnian Bay. Decreases in fecundity concurrent with lesions of the female reproductive organs were also found in both species of seals (Bäcklin, Bredhult & Olovsson, 2003). No prevalence of uterine fibroids was reported in grey seals outside the Baltic Sea. Some types of PCBs were subsequently shown to have proliferative effects on cells in the middle layer of uterine wall (myometrium) of the seal in vitro, thus suggesting that PCBs could take part in the growth of the uterine fibroids (Bäcklin, Eriksson & Olovsson, 2003), as also observed in rodent models of humans (see Chapter 2.2.2.4).

More recently, O'Hara & Becker (2003) increased the weight of evidence for chemical causation of fibroids with a very thorough study of grey seals during a period when organochlorine concentrations were decreasing in the Baltic. Decreasing organohalogen concentrations in seals were associated with a reduction in uterine obstructions and an increase in pregnancies, providing additional evidence of a causal link between EDCs and fibroids in the grey seal.

It is also noteworthy that reproductive success among other pinnepid species has become a matter of recent concern. Several Alaskan populations of northern fur seal, the Galapagos sea lion (Alava et al., 2009) and the Steller sea lion (Trites & Donnelly, 2003) have experienced recent declines, attributed to reduced pupping rates. The causes and timing of these reproductive failures are unknown, but in the fur seal are suspected to be linked to bioaccumulation of environmental contaminants in maternal body tissues (e.g. Beckmen et al., 2003; Towell, Ream & York, 2006). Moreover, in the contaminated St. Lawrence estuary (Canada) fibroids were recorded in 8 of 12 adult female beluga whales examined from 1996 to 1998 (Mikaelian et al., 2000). Also in other marine mammals, fibroids, as well as ovarian tumours (dysgerminoma), were observed in 5% of a large sample (n=502) of dusky dolphins caught off of Peru in 1985-87 and 1992-1994 (Van Bressem et al., 2000). Of 11 mature females with ovarian tumours or cysts or uterine tumours, only one (9.1%) was pregnant; this is significantly less than the expected pregnancy rate (53.3% in a random sample of Peruvian dusky dolphins).

Recently, in a European Commission funded study (Murphy et al., 2010), the relationship between PCB exposure and female reproductive health was examined in European harbour porpoise and in the short-beaked common dolphin. High PCB concentrations were reported in immature porpoise, of which 42% had contaminant loads above a threshold level of 17 µg/g lipid for adverse health effects based on experimental studies of both immunological and reproductive effects in seals, otters, and mink. Interestingly, all pregnant porpoise sampled had contaminant loads below 20 µg/g lipid compared with much higher levels in resting (not pregnant or lactating) mature females. Moreover, dolphins with the highest PCB burdens (and above the threshold level) were also resting mature females. These individuals also had the highest number of ovulatory scars on their ovaries. This suggests that, due to high contaminant burdens, female common dolphins may be unable to reproduce and, thus, continue ovulating; or females are not reproducing for some other reason, either physical or social, and are accumulating higher levels of contaminants in their blubber because they have fewer loss mechanisms (such as lactation). The high associated PCB burden may thus be either (or both) the cause of infertility or the consequence of infertility. In contrast, in harbour porpoises, once the effect of age and nutritional condition were taken into account, the data so far suggest that higher POP concentrations (PCB, HBCDD and DDE) tended to be associated with lower numbers of corpora scars, possibly indicating that high contaminant levels were inhibiting ovulation. However, as discussed earlier with the seal studies, associations between contaminant loads and reproductive problems found in adult female marine mammals of breeding age could easily be confounded by the fact that much of this contaminant load is transferred to the offspring during lactation. Therefore, lower contaminant loads found in breeding females could simply be reflective of this maternal transfer, rather than any true causative association with the reproductive status of these females.

2.2.3.2 Non-mammalian vertebrates

Examples of female reproductive system disorders, occurring concomitantly with chemical exposure, can be provided for several non-mammalian vertebrate classes. Perhaps one of the most reported cases is that of a reptile exposed to high concentrations of pesticides (dicofol (a pesticide chemically related to DDT), DDT itself and metabolites DDD and DDE) following an accidental spill into a tributary of Lake Apopka (Florida, USA) in 1980, which is reviewed in Guillette, & Crain (2000). This had a profound effect on the resident American alligator population. Although not seen in any of the other Florida lakes studied, there was a dramatic decline in juvenile recruitment in Lake Apopka alligators. Moreover, Guillette and colleagues reported Apopka female alligators with abnormal ovarian morphology, large numbers of polyovular follicles and polynuclear oocytes (Milnes & Guillette, 2008; Hamlin & Guillette, 2011) and plasma estradiol concentrations almost twice as high as those in female alligators from a reference lake.

Laboratory studies supported the hypothesis that contaminant exposure was the most likely explanation for the reproductive abnormalities in female alligator from Lake Apopka, and that these effects would compromise the reproductive capabilities of wild animals (Milnes & Guillette, 2008; Hamlin & Guillette, 2011). Multiple eggs per follicle and abnormal hormone levels can be induced in alligators by embryonic exposure to hormonally-active pesticides. Other estrogenic chemicals, such as DES, have also been shown to cause multi-oocyte follicles in laboratory mice (Guillette & Moore, 2006). In both alligators and mice, adult reproduction appears to be impaired by this condition and, in women, multi-oocyte follicles (MOFs) or polyovular follicles are associated with diminished in vitro fertilisation success and increased early miscarriage as well as with ovarian teratomas (a type of ovarian tumour present from birth; Guillette & Moore, 2006). Gene expression profiles of ovaries from neonates hatched from eggs collected on Lake Apopka are similar to profiles seen in laboratory animal models and women with premature ovarian failure and PCOS (Moore et al., 2010a; 2010b; 2011).

Taken together, data indicate that more research is needed to understand the role endocrine disruptors might play in causing MOF formation, as well as other reproductive disease states in humans and wildlife. Test methods for this endpoint have not been developed.

Reproductive endocrine disruption has also been reported across a range of bird species since the 1950s. Historically, investigations centred on the issue of eggshell thinning in predatory birds in relation to organochlorine pesticide exposure (Ratcliffe 1967; 1970; Lundholm 1997), which ultimately prompted the ban on the use of DDT, in particular, in North America and Europe. Although this led to a reduction in body burdens in birds and an improvement in eggshell thickness, with the subsequent recovery of many of the affected populations, other compounds linked to reproductive failure, such as dioxins and polybrominated diphenylethers (PBDEs), continue to be found at elevated concentrations in wildlife, especially near

urban centres (see Chapter 3.2.1.3). Some are very persistent, bioaccumulating to levels capable of causing toxic effects in predatory species (Bosveld & Van Den Berg, 2002). For example, American kestrels fed a PBDE-contaminated diet had delayed times to egg laying, eggs with thinner shells and reduced weights, and fewer hatchlings (Fernie et al., 2009). In addition to raptors, adverse effects on black-crowned night herons, herring gulls, double-crested cormorants and common terns (Grasman, Scanlon & Fox, 1998) have been reported. Adverse effects of PBDEs include eggshell thinning, embryonic deformities of the foot, bill and spine as well as chick death and retarded growth (e.g. Bowerman et al., 2000). These effects could be directly related, and chick survival inversely related, to the concentration of the contaminants present (Burgess & Meyer, 2008). The effects reported in the field are supported by data generated in the laboratory, which have revealed that estradiol administration during early development can cause reproductive impairments in adulthood, such as the retention of ovulated yolks within the body cavity, egg shell thinning and reductions in the number of eggs laid and in egg length and width. The production of thinner shells is thought to be at least partly due to reductions in the shell gland expression of carbonic anhydrase, an enzyme essential for the formation of the egg shell (Brunström et al., 2009; Rochester et al., 2008).

The masculinization and feminization of male fish living near the outfalls of paper mills and some sewage treatment works (STW) plants is a well known phenomenon that appears to be consistent across investigators, geographical regions, species and habitats (Tyler & Jobling, 2008). Although the effects of estrogenic sewage effluent exposure are most widely reported in male fish, many laboratory and several field studies clearly show that estrogen exposure is also consistent with effects in females, including reduced fecundity, alterations in the timing of sexual maturity and reproduction and premature atresia (or degeneration and reabsorption of preovulatory follicles; reviewed in Tyler & Jobling, 2008; Jobling et al., 2002). Masculinizing effects of pollution on female fish, though apparently less prevalent, have also been reported. In fact, the first evidence of ED in fish was provided by the discovery of masculinized female mosquitofish living in a stream receiving pulp mill effluent (Howell, Black & Bortone, 1980), an observation that has been confirmed in other countries (e.g. Larsson, 2000). Another source of effluent, that of intensive animal husbandry, has also been shown to masculinize wild fish due to the presence of anabolic steroids in the effluent (Orlando et al., 2004). Furthermore, the masculinization of females has also been reported in developing fish exposed to aromatase inhibitors, such as fadrozole (Fenske & Segner, 2004). This is of potential significance due to the widespread use of azole derivatives in agriculture. It is important to recognize, however, that masculinization can also be induced in fish in response to other, non-chemical stressors such as hypoxia, as demonstrated in the Atlantic croaker (*Micropogonias undulates*) (Thomas and Rahman, 2012). This highlights the need for caution when interpreting data from field-based studies.

2.2.3.3 Invertebrates

Compared with vertebrates, little is known about the manifestation of endocrine disrupting effects on the reproductive system of female invertebrates. However, there are some historical reports in which females have exhibited signs of masculinization, apparently in association with exposure to EDCs. For example, it has been shown that potent androgen receptor (AR) agonists and aromatase inhibitors, as well as tributyltin (TBT), induce imposex in prosobranch female snails, a condition in which the penis "imposes" on the normal female reproductive anatomy. The associated development of the sperm duct (vas deferens) can, in extreme cases, lead to blockage of the oviduct of the female, resulting in sterility and population declines (Gibbs & Bryan, 1986). The effects of TBT are the best known example of endocrine disruption in invertebrates, the occurrence of which has been reported in a number of gastropod molluscs (Ellis & Patissina, 1990; Titley-O'Neal, Munkittrick & Macdonald, 2011).

Although the effects of TBT on the reproductive system of female gastropods are well established, the underlying mechanisms are not yet understood. Since testosterone itself appeared to induce imposex in some species, it was suggested by various authors (Spooner et al., 1991; Bettin, Oehlman & Stroben, 1996; Santos et al., 2002) that TBT and testosterone may competitively inhibit P450 aromatase activity, thereby preventing the conversion of androgens to estrogens (and consequently increasing testosterone levels) or by inhibiting testosterone excretion or decreasing the esterification of testosterone (Gooding et al., 2003). Whilst androgens have been identified in several mollusc species, attempts to isolate an androgen receptor from molluscs have so far been unsuccessful and so the mechanism through which these apparent "androgenic" effects are occurring is unknown and controversial (Sternberg, Hotchkiss & Leblanc, 2008). Indeed, the only convincing body of evidence for mechanisms of masculinization in molluscs indicates that tributyltin-induced imposex involves the abnormal modulation of the retinoid-X receptor (RXR; Nishikawa et al., 2004; Kanayama et al., 2005; Castro et al., 2007; Lima et al., 2011), also known to be involved in male reproductive differentiation and external genitalia formation in mice (Kastner et al., 1996; Ogino et al., 2001).

In addition to the masculinization of female gastropods, there is also some laboratory-based evidence to suggest that EDC exposure may be associated with a phenomenon known as "superfeminization". Oehlmann et al. (2000) reported that the exposure of ramshorn snails to BPA induced a superfeminization syndrome at all concentrations tested (from 1-100ug/L BPA). Super-females were characterised by additional sex organs, enlarged accessory sex glands, gross malformations of the pallial oviduct, enhanced egg production outside the main spawning season, and increased female mortality. Effects were concentration dependent (except for mortality) and statistically significant at every test concentration. Limitations on this first study included the lack of analytical confirmation of the exposure regimes, a lack of replication, and the absence of

a positive control. However, two follow-up exposures with expanded experimental and quality assurance procedures confirmed the original findings (Schulte-Oehlmann et al., 2001; Oehlmann et al., 2006) and were also able to demonstrate the importance of the stage of the reproductive cycle and water temperature in the outcome of such studies. Nevertheless, the effects reported by Oehlmann and colleagues have not been reported by other laboratories performing similar, but not identical, studies (e.g. Forbes et al., 2007a; 2007b), leaving the important question of whether or not BPA is likely to cause adverse effects on molluscs at concentrations that are widely reported in the aquatic environment.

Taken together, it would appear that, in all of the wildlife cases of endocrine disruption of the female reproductive tract, there is some evidence to support chemical causation of the disorders. Moreover, there are extensive laboratory-based data that support the chemical causation of these female reproductive abnormalities. In this respect, the developmental exposure of fish to estrogenic chemicals has been associated with delayed reproductive development, aberrations in gonad morphology and increases or decreases in fecundity, depending on the dose and timing of the exposure. Whilst these effects may not be classified as "disorders", but rather the manifestation of physiological perturbations, they may still significantly impact upon female reproductive capacity and success.

2.2.3.4 Interspecies extrapolations

It is important to recognize that some female reproductive disorders are not comparable across all species. Using endometriosis as an example, it is clear that whilst widely reported in humans and many other mammals, this condition is not universally applicable to all species. All vertebrates, reptiles, fishes, birds or amphibians, except some teleost fishes, have well developed uterine and tubal structures derived embryologically from the Müllerian duct, but they do not exhibit the same degree of endometrial growth. Thus the symptoms of endocrine disruption may be deceptively different in these species compared with mammals, although the underlying mechanisms may be the same.

Alternatively, it is important to note that, due to the greater reproductive plasticity observed in wildlife, additional endpoints may be affected that have no clear analogy in humans. However, these symptoms could still form part of the same underlying syndrome and, thus, have the capacity to inform our overall understanding of reproductive dysfunction in other forms of wildlife, as well as in the human population.

2.2.4 Main messages

- Recent studies indicate that a number of female reproductive disorders that can impair fertility or fecundity are prevalent in some human populations.
- Evidence from animal studies with rodents indicates that exposure to endocrine disrupting chemicals during gestation can lead to reproductive health problems in female offspring, as their eggs are exposed whilst they are developing.
- Increased understanding of endocrine pathways governing female reproductive processes suggests that a role for EDCs in the multi causality of female reproductive disorders is biologically plausible.
- There is limited and conflicting experimental and epidemiological evidence to support a role for EDCs in premature puberty and breast development and in causing fibroids (PCBs) and endometriosis (phthalates and dioxins), and almost no evidence for causation of PCOS or infertility; few studies have, however, examined chemical causation of these diseases directly and very few chemicals have been investigated. There is limited evidence that potential endocrine disruptors such as mercury and cadmium, and organochlorine and organophosphate pesticides are associated with increased risk of miscarriage, preterm delivery and reduced fetal growth.
- There are not enough historical data to state whether or not the incidence of the many disorders of female reproductive function are correlated with one another, and therefore no obvious suggestion of a common etiology. It is clear that alterations in steroid hormone levels (principally estrogens) during prenatal life are critical factors.
- Symptoms similar to those seen in women occur in a variety of wildlife species and have been linked to exposure to contaminants (particularly PCBs and organochlorine pesticides) in some cases, e.g. fibroids in seals.
- Vertebrate wildlife are important sentinels for women's reproductive health, as the hormonal control and underlying genetic and cellular responses of female reproduction are well conserved amongst the vertebrates. Further, wildlife species often live in direct contact with similar or the same complex mixtures of anthropogenic environmental contaminants to which humans are exposed.
- There are many gaps in our knowledge of endocrine disruption of the female reproductive system. Many of the mechanisms are poorly understood and the number of chemicals that have been investigated for endocrine disruption in females is limited.
- There are many gaps in the available test methods for screening chemicals for endocrine disrupting effects on female reproduction. Regulatory tests for many wildlife taxa are currently not developed and in mammalian assays, endocrine endpoints measured are sometimes not adequate to detect possible roles of endocrine disrupting chemicals in inducing many of the female reproductive disorders and diseases described here.

2.2.5 Scientific progress since 2002

Since the IPCS (2002) was published, major advancements in our knowledge of endocrine disruption have occurred. These include:

- Downward trends in the age at breast development in Europe to substantiate the USA data.
- Greater understanding of endocrine pathways governing female fecundity and fertility, the timing of puberty, and regulation of menopause.
- Increasing experimental evidence that chemicals can interfere with endocrine signaling of pubertal timing, fecundity and fertility and with menopause.
- There are more suggestions that consequences of maternal exposures during fetal life might influence birth outcomes.
- More evidence linking phthalate exposure to endometriosis.
- Subtle effects of chemicals may only be seen when combined with other risk factors such as genetic susceptibility, age, alcohol consumption and smoking.
- More evidence now exists that reduced reproductive success in female seals, birds, alligators and gastropods are related to exposure to several persistent organic pollutants. When exposure to these EDCs decreases, reproductive effects in wild populations also decline.
- A wide variety of assays have been developed for the study of endocrine disruption. Many of these could be turned into validated regulatory tests to cover aspects of the endocrine system that are outside current testing strategies. Some modifications to existing tests have been made but even the latest of these has considerable gaps with respect to covering endpoints relevant for the detection of endocrine disrupting chemicals.

2.2.6 Strength of evidence

Female reproductive disorders are prevalent in some wildlife and human populations. In humans, they are a major cost to the health-care services in which they have been studied. These diseases include polycystic ovary syndrome (PCOS), fibroids, endometriosis, premature ovarian failure, and disorders associated with poor pregnancy outcomes. There is also sufficient evidence that breast development occurs earlier in girls in the USA and Europe. In some wildlife species, there is sufficient historical evidence that female reproductive disorders were caused by exposure to endocrine disrupting persistent organic pollutants (e.g. imposex in gastropods, fibroids in seals, and egg shell thinning in raptors) and associated with population declines. It is possible that these population-level effects are associated with adverse ecological consequences and that other emerging endocrine disrupting chemicals play a role in the causation of current diseases and disorders and declines in biodiversity in wildlife (see also Chapter 2.12).

Although it is likely that there is a role for endocrine disrupting chemicals as causal factors of female reproductive disorders in humans, the actual experimental and epidemiological evidence to support this hypothesis is limited for puberty/breast development, fibroids and endometriosis, and insufficent for PCOS, infertility and irregular menstrual cycles. In Africa where spraying of DDT is done in homes for prevention of malaria, although studies are conflicting, the WHO concluded that the combined human and animal data available do raise concern for effects of exposure to DDT on female reproductive health, albeit further data are needed (IPCS, 2011). The case for chemical causation of fibroids and of infertility/reduced fecundity, in particular, are made stronger by the existence of wildlife populations with reduced pupping rates and high rates of fibroids inhabiting particularly polluted aquatic environments, and of the recovery of these populations following the decline in the concentrations of the suspected chemical causes (e.g. PCBs and organochlorine pesticides). In addition, laboratory experiments provide sufficient evidence that a range of these types of chemicals can cause these effects. Moreover, for endometriosis, there is limited evidence to support the hypothesis that exposures to PCBs and/or phthalates are an important part of the multifactorial etiology of this disease.

Taking the wildlife and human evidence together, it is likely that exposure to PCBs and perhaps also other endocrine disrupting chemicals (e.g. phthalates and dioxins) that act in the same/similar ways play a role in the causation of fibroids and endometriosis in humans, and it is biologically plausible that for their involvement in PCOS, early age at menarche, and/or breast development and irregular menstrual cycles. In some populations of wildlife, a role for EDCs in causing fibroids is very likely or certain.

2.2.7 References

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2.3 Endocrine disrupting chemicals and male reproductive health in humans and wildlife

2.3.1 Overview of male reproductive health trends in humans and wildlife and evidence for endocrine disruption

Male reproductive health has been a major focus of research on endocrine disrupting chemicals (EDCs) since the early 1990s, when evidence of adverse secular trends in sperm counts first came to light. Subsequent research also showed similar trends in other male reproductive and developmental abnormalities that appeared to have occurred in a manner concomitant with the rapid expansion of the chemical industry, and the associated release of thousands of anthropogenic chemicals into the environment. This has led to growing speculation that chemicals with endocrine disrupting properties may be partially responsible for the decline in male reproductive health over the past several decades. The following diseases and disorders are suspected to be caused, at least partially, by exposure to EDCs during early life:

- **Testicular Germ Cell Cancers (TGC)** have been increasing over the past 40 to 50 years (by as much as 400%) in the majority of industrialised countries, where they are the most common cancers in young men aged 20-45 (Huyghe, Matsuda & Thonneau, 2003; Richiardi et al., 2004). The distinct increase in TGC over a couple of generations is suggestive of environmental causation, although genetic causes may also play a role.
- **Congenital Cryptorchidism:** The incidence of congenital cryptorchidism (where one or both testes do not descend into the scrotum) varies between 1-9 % and has increased in many countries (Toppari et al., 2010) The intra-abdominal temperature is toxic for germ cells, and so cryptorchidism often leads to infertility and is associated with increased testicular fibrosis. It is also associated with an increased risk of testicular cancer later in life.
- **Hypospadias:** This is a birth defect in which the urethra opens on the underside of the penis instead of the tip. Its incidence has increased in several regions of Australia, Europe, and the USA (Källen et al., 1986; Paulozzi, 1999; Toppari, Kaleva & Virtanen, 2001; Nassar, Bower & Barker, 2007; Lund et al., 2009), although its rate is commonly underestimated because of problems with clinical diagnosis and reporting (Toppari, Kaleva & Virtanen, 2001).
- **Semen Quality:** Prospective studies on the general population of Nordic, Baltic, German, Spanish and Japanese men show that 20-40% of young men have semen quality below what andrologists would consider to be compatible with good fecundity (below 40 mill/mL) (Bonde et al., 1998; Guzick et al., 2001; Skakkebæk, 2010). Whilst this cut off differs from the WHO reference value of 15 mill/mL, the fecundity of a man decreases steeply with sperm concentrations below 40 mill/mL, and men with sperm counts below 40 mill/mL would be sub fertile, even though conception could well occur, particularly in the 15-40 mill/mL range.
- **Testicular Dysgenesis Syndrome:** Testicular Germ Cell Cancer is often found in association with hypospadias, cryptorchidism and low semen quality, suggesting that they are risk factors for one another (Boisen et al., 2004; Jacobsen et al., 2006) and that they could be related components of a single underlying condition, termed 'testicular dysgenesis syndrome' (TDS), originating during fetal life (Skakkebæk et al., 2007; Møller & Skakkebæk, 1999) and caused by exposure to contaminants. Some experimental and epidemiological data now exist to support this hypothesis.

Alongside the evidence of continued adverse trends in male reproductive health in humans, data describing patterns of reproductive dysfunction in male wildlife have also expanded considerably over the past ten years. In many cases, these patterns appear to mirror those observed in humans, in that the affected wildlife populations exhibit a suite of symptoms consistent with a demasculinizing and/or feminizing mode of action. There are several examples (some closely studied) of male reproductive system disorders in wildlife:

- **Cryptorchidism, infertility and abnormal antler formation in large numbers (68% in some populations) of Sitka black-tailed deer on Kodiak Island, Alaska** (Bubenik & Jacobson, 2002) and in white-tailed deer sampled in the Bitterroot Valley of west-central, Montana, U.S.A. between 1996-2000 (Hoy et al., 2002). Although genetic causes cannot be ruled out completely, a recent analysis of the Sitka deer favours exposure to environmental contaminants as a likely contributing cause (Latch et al., 2008).
- **Reduced blood testosterone levels and testis size in polar bears** in East Greenland (Sonne et al., 2006) and Svalbard (Oskam et al., 2003). These problems have been correlated with tissue concentrations of persistent organic pollutants (POPs).
- **Reduced phallus size, changes in the structure of the testes, and depressed testosterone concentrations in American alligators** exposed to high concentrations of pesticides (the DDT-like pesticide dicofol, DDT itself and its metabolites DDD and DDE) following an accidental spill into a tributary of Florida's Lake Apopka in 1980 (reviewed in Guillette et al., 2000).
- **Ovarian tissue in the testes of male birds** in a breeding colony of terns on Bird Island, Massachusetts (USA); the prevalence of this intersex condition was associated with PCB and dioxin levels in the developing eggs (Hart et al., 1998).

- **Relationship between EDC burden and depressed testosterone levels in the eggs of wild birds** (Arctic-breeding glaucous gulls; Verboven et al., 2008).
- **Intersex in several species of frogs and toads correlated, in some cases, with exposure to various pesticides in agricultural areas** (Reeder et al., 2005; Hayes et al., 2003; McDaniel et al., 2008; McCoy et al., 2008).
- **Feminization of male fish living near sewage treatment works (STW) in many geographical regions of the world, species and habitats** (Cheek, 2006). Feminized male fish have reduced testosterone levels, feminization of external genitalia (e.g. Jobling et al., 2002a) and are less successful than normal males in competitive spawning experiments (e.g. Harris et al., 2011).
- **Intersex in the estuarine bivalve, *Scrobicularia plana*, in UK estuaries:** varying degrees of intersexuality were reported in over 20% of individuals sampled from 17 out of 23 populations and this has been putatively linked with exposure to EDCs (Chesman & Langston, 2006).
- **Data from many laboratory-based studies also support the chemical causation of testicular abnormalities in wildlife.**

The apparent parallels between the effects reported in humans and in various wildlife populations are not surprising given that there is often considerable overlap between their environments and food chains, as well as between the endocrine control of the male reproductive system. Based on a review of currently available data, a common causative mechanism for human TDS and feminization in vertebrate wildlife is hypothesised. In studying endocrine disruption in wildlife, we may begin to understand the effects of endocrine disrupting chemicals on human development within the same framework.

Hormonal mechanisms underlying male reproductive disorders and diseases

In all vertebrates, and some invertebrates, both the differentiation and development of the male reproductive system, including the differentiation of the Wolffian duct system and the masculinization of the external genitalia, are under the influence of the androgen testosterone. In rodents, Carruthers & Foster (2005) and Welsh et al. (2008) recently showed that there is a "male programming window", when the fetal testes begin to synthesise testosterone, during which the entire programme of development of the male reproductive tract is set up. It is during this time that the distance between the anus and the genitals (the so-called anogenital distance; AGD; shorter in females than in males) is fixed for life. This period is also essential for proliferation of Sertoli cells, often thought of as the conductors of the orchestra of other testis-specific cells in the testis in all animals. Sertoli cell number is determined and, in turn, determines the final sperm count of a male during adult life (see Sharpe, 2010).

Testosterone is synthesised by cells within the testes and adrenal glands. Local concentrations of testosterone are also modulated within target tissues as a result of expression of enzymes such as aromatase (which converts testosterone to estradiol). Estrogenic steroid hormones also regulate certain functions of the male reproductive system important for the maturation of sperm, and may be necessary for a healthy libido. Any disturbance in androgen action or reduction in its production has demasculinizing effects. If there is no functional androgen receptor, for example, androgens cannot relay their signals to developing organs and systems, such that genotypic males develop into phenotypic females.

It is, therefore, biologically plausible that EDCs that mimic or interfere with the action or synthesis of these hormones could play a central role in the causation of disorders associated with declining male reproductive health.

Deregulation of androgen production or action causes male reproductive disorders

Mechanistic evidence suggests that a proportion of male reproductive endocrine disorders are caused by male hormone (e.g. androgen) insufficiency and/or by an imbalance between female and male hormones during critical times during the life cycle (e.g. when the testes and genitalia are differentiating and/or during puberty when the organs are maturing). This can lead to malformations such as cryptorchidism and hypospadias, as well as changes in anogential distance. Indeed, it has recently been proposed that exposure to endocrine disrupting chemicals may cause this imbalance and cause adverse effects which may only become evident later in life. Furthermore, these disorders commonly occur simultaneously, which prompted Skakkebæk, Rajpert-De Meyts & Main (2001) to hypothesise that the increasing frequency of this suite of effects in the human population in recent decades may reflect a single underlying condition, termed TDS.

The concept of TDS is based on the premise that the associated symptoms have a common origin in fetal development, and that the extent and severity to which they are manifested is dependent on the degree to which normal developmental processes have been perturbed. In addition, it assumes that any perturbations occurring during the male programming window are irreversible and have lifelong implications for the affected individual and, potentially, also for their offspring. Although there is good evidence that each of the diseases comprising TDS have strong genetic components, Skakkebæk, Rajpert-De Meyts & Main (2001) noted that the majority of baby boys born with these symptoms lacked the expected genetic aberrations, indicating that environmental factors must play an important role in the etiology of these phenomena. Hormonal perturbations, arising from EDC exposures, have been widely implicated in the causation of TDS in humans, and also in the widespread reports of reproductive dysgenesis in wildlife, between which there are obvious parallels, as well as clear distinctions.

Animal studies suggest endocrine disrupting chemicals could cause TDS

In rat studies, fetal exposure to the phthalate plasticizers diethyl hexyl phthalate or dibutyl phthalate, to various chemicals with androgen receptor antagonist properties (e.g. the fungicides vinclozolin, procymidone or prochloraz), or to chemicals that interfere with steroidogenic enzymes (e.g. finasteride, prochloraz, linuron) causes disturbances of testosterone production and results in hypospadias (termed cleft phallus in rodents), cryptorchidism, impaired fertility and dysgenetic testicular histology a TDS-like outcome (Fisher et al., 2003; Foster, 2005; Foster, 2006; Gray et al., 2006; Hass et al., 2007, Wilson et al., 2008). Other chemicals with estrogenic activity cause similar effects, as do prostaglandin synthesis inhibitors, such as paracetamol (Kristensen et al., 2010). Furthermore, these compounds act in an additive fashion when occurring together (Hotchkiss et al., 2010; Rider et al., 2010; Christiansen et al., 2009). There is a large and convincing body of literature on adverse effects of EDCs on the male reproductive system in rat models, some of which is compiled in section 1 of this chapter, **Table 2.1.**

The next section presents an overview of male reproductive endocrine diseases and disorders seen in humans, followed by some relevant examples taken from the extensive wildlife literature, which are discussed in terms of the evidence to support the case for causation of male reproductive disorders by EDCs. For information on the types and levels of EDCs found in wildlife and human tissues, see Chapter 3.2.1 & 3.2.2, respectively. Use of the chemicals is also described in Chapter 3.1.

2.3.2 Evidence for endocrine disruption of the male reproductive system in humans and in mammalian models of humans (rodents and primates)

2.3.2.1 Testicular germ cell cancer (TGC)

In countries with cancer registries since the middle of the 1900s, the incidence of TGC can be seen to have increased up to 400% (see **Figures 2.2-2.4**). Moreover, remarkable differences in TGC incidence have been demonstrated between different countries, with the highest rates seen in countries with Caucasian populations and high activities

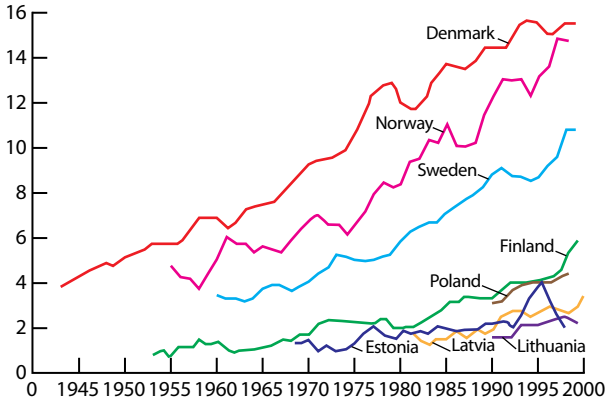

Figure 2.2. Trends in incidence of testicular cancer in northern Europe. Age-standardized (World standard population) by year of diagnosis and country. From Richiardi et al. (2004; Used with publisher's permission).

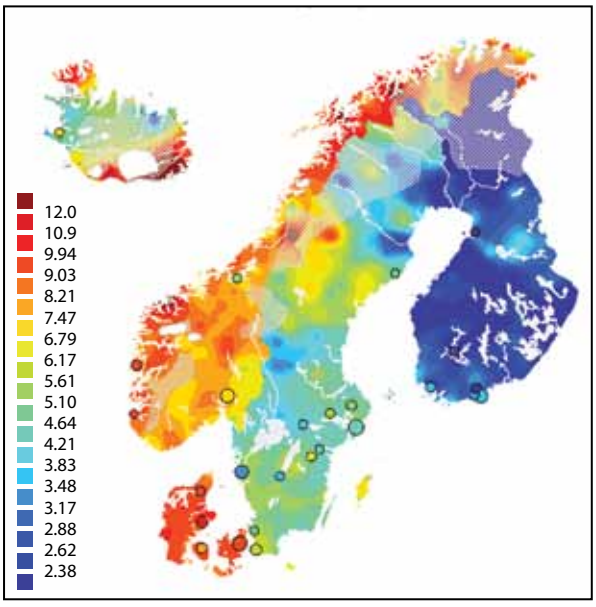

Figure 2.3. Heat map showing the incidence (per 100 000) of testicular cancer in nothern Europe, 1989-1994. The "hotter" the colour, the greater the incidence of testicular cancer. Source: *Finish* Cancer Registry 21.06.2007.

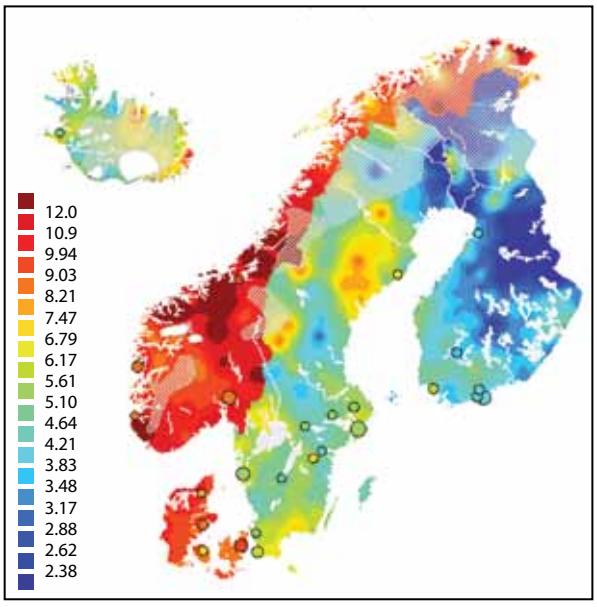

Figure 2.4. Heat map showing the incidence (per 100 000) of testicular cancer in nothern Europe, 1998-2003. The "hotter" the colour, the greater the incidence of testicular cancer. Source: *Finish* Cancer Registry 21.06.2007.

in the industry and farming sectors, including Switzerland, Denmark and New Zealand. Recently, very high rates of TGC have also been documented in Norway and Chile (Jacobsen et al., 2006; Chia et al., 2010). In contrast, low incidences and smaller increases in TGC rates have been seen in countries with Asian populations (Bray et al., 2006) and in countries with mixed populations, where African Americans have much lower incidences of TGC than Caucasians living in the same areas. Thus, although the increase in TGC over a couple of generations is suggestive of environmental causation, genetic susceptibility genes clearly have some involvement in its etiology. Increasing rates of TGC are of special interest to this review as they are also associated with impaired semen quality (Jacobsen et al., 2000) and lower fertility, even prior to the development of cancer (Møller & Skakkebæk, 1999).

Mechanisms of TGC

Although the etiology of TGC is unknown, there is abundant evidence that carcinoma in situ testis (CIS), which is a precursor for all types of TGC, is generated during fetal development; in other words TGC seems to have a prenatal origin (Rajpert-De Meyts, 2006). Numerous studies have shown that the precursor cells of TGC, the CIS cells, are like the primitive reproductive cells of the embryo (the gonocytes or primordial germ cells; PGCs) and share gene expression factors with embryonic stem cells. A current hypothesis is that the CIS develops because the somatic compartment of the fetal testis (composed of Sertoli and Leydig cells) fails to drive the normal differentiation of the primordial germ cells into spermatogonia (Rajpert-De Meyts, 2006; Looijenga et al., 2011). Such failures of Sertoli and Leydig cells during development may not only result in TGC, but also in spermatogenic disorders, cryptorchidism, hypospadias and other disorders of sexual development (see later). In all these disorders, besides CIS cells, other persisting testicular changes reflecting dysgenesis have been described (Chemes, 2001; Høi-Hansen et al., 2003; Skakkebæk et al., 2003). A hypothesis that maternal hormone levels were associated with TGC in sons was first presented in the 1970s by Henderson and his group (Henderson et al., 1979).

Epidemiological evidence for EDCs causing testis germ cell cancer

Follow up studies on the sons of women exposed to the synthetic estrogen diethylstilbestrol during pregnancy (see section 2.1) have indicated that they have a slightly increased risk of developing TGC (Strohsnitter et al., 2001). The hypothesis that maternal exposures could be important for development of TGC was also supported by epidemiological studies from Scandinavia showing that men born during World War II had a decreased risk of developing TGC as they grew up as compared with those born either before or after the war (Bergstrom et al., 1996). The hypothesis that internal sex hormone action plays a role in the pathogenesis of TGC is supported by a recent study showing that baldness, acne and increased androgen levels during puberty were negatively associated with development of TGC (Trabert et al., 2011).

Relating maternal exposures to tumours occurring 20-40 years later is a difficult task (Cook, Trabert & McGlynn, 2011) and no consistent results have indicated that external exposures postnatally are associated with TGC. A single epidemiological study found that prenatal exposures to POPs via the mother, was a risk factor for TGC, although they did not find evidence for links between postnatal exposures to POPs and TGC (Hardell et al., 2006). Whilst there is little doubt that the increase in TGC incidence during the last half century is linked to environmental factors, the possible roles of EDCs remain to be determined.

2.3.2.2 Cryptorchidism

The incidence of congenital cryptorchidism, a condition in which one or both testes are not located at the bottom of the scrotum at the time of birth, varies between 1% and 9% according to cohort studies (Toppari et al., 2010). Accurate diagnosis of cryptorchidism requires careful clinical examination. Prospective clinical studies and registry-based studies can give very different incidence figures because the latter ones usually pick up only those who have been operated on, and this introduces yet another confounding factor; a large proportion of operated boys have had so-called acquired cryptorchidism, i.e. their testes have been descended at birth, but ascended during childhood (Hack et al., 2003; Wohlfahrt-Veje et al., 2009). Despite the difficulties with diagnosis, over the last few decades there is clear evidence that the incidence of cryptorchidism has increased in Denmark (Bueman et al., 1961; Boisen et al., 2004) and the UK (Scorer, 1964; Group, 1992; Acerini et al., 2009). In a joint Danish-Finnish cohort study, Denmark showed a 3-4 fold higher birth rate of cryptorchidism than Finland (Boisen et al., 2004). The reasons for these trends are not known, although it is apparent that environmental factors play an important role. **Table 2.3** includes incidence data from prospective cohort studies on congenital cryptorchidism and **Figure 2.5** highlights the increasing trends.

Hormonal mechanisms underlying cryptorchidism

Testicular descent is regulated by two hormones, testosterone and insulin-like peptide 3 (INSL3), that are secreted by the Leydig cells in the testis. INSL3 stimulates development of the gubernaculum that attaches the testis close to the inner opening of the inguinal canal. During late gestation the testes migrate through the inguinal canals to the scrota. This is critically dependent on normal androgen action. When there is a lack of androgen action (insensitivity or defective androgen production), the gonads remain either in the abdomen or in the inguinal canals. Therefore disruption of either INSL3 or testosterone production or action can cause cryptorchidism.

Mutations in the androgen receptor gene, steroidogenic enzymes needed for androgen production, or hypothalamic-pituitary regulators needed for testicular stimulation are rare reasons for cryptorchidism (Virtanen et al., 2007; Barthold, 2008). Mutations in *INSL3* and its receptor, *RXFP2,* have been

Table 2.3 Rate of congenital cryptorchidism in prospective clinical studies using clearly defined criteria of cryptorchidism.

Country	Reference	Number of subjects	Diagnosis based on	Rate of cryptorchidism at birth
USA, Rochester, Minnesota, St. Mary's Hospital	(Harris & Steinberg, 1954)	n=4474	position (testis cannot be manipulated into the scrotum)*	1.3% (BW>2500g), 1.5% of all boys
Denmark, Copenhagen, Rigshospitalet	(Buemann et al., 1961)	n=2701	position	1.8% (BW>2500g), 4.1% of all boys
UK, West London, Hillingdon Hospital	(Scorer, 1964)	n=3612	distance measurement	2.7% (BW>2500g), 4.2% of all boys
India, Kanpur, Dufferin Hospital and U.I.S.E Maternity Hospital	(Mital & Garg, 1972)	n=2850	distance measurement	1.6% of full-term single born boys
Taiwan, Provincial Tao-Yuan Hospital	(Hsieh & Huang, 1985)	n=1208	position (presence or absence of testes in the scrotum)*	4.1% in preterm boys, 1.4% in mature boys
Korea, 38 hospitals	(Choi et al., 1989)	n=7990	position	0.7% of all boys
UK, Oxford, John Radcliffe Hospital	(Group, 1992)	n=7400	position distance measurement	3.8% (BW≥2500g), 4.9% of all boys (excluding boys with severe congenital malformations) 4.1% (BW≥2500g), 5.0% of all boys (excluding boys with severe congenital malformations)
USA, New York, Mount Sinai Hospital	(Berkowitz et al., 1993)	n=6935	distance measurement	2.2% (BW≥2500g), 3.7% of all boys
Malaysia, Kuala Lumpur, University Hospital	(Thong, Lim & Fatimah, 1998)	n=1002	position	2.4% (BW≥2500g), 4.8% of all boys
Italy, Pisa, S. Chiara Hospital and Division of Neonatology at the University of Pisa	(Ghirri et al., 2002)	n=10730	position	3.5% (BW>2500g), 6.9% of all boys
Denmark, Copenhagen, Rigshospitalet	(Boisen et al., 2004)	n=1046	position	8.4% (BW>2500g), 9.0% of all boys
Finland, Turku, Turku University Hospital	(Boisen et al., 2004)	n=1455	position	2.1% (BW>2500g), 2.4% of all boys
Lithuania, Panavêžys City Hospital	(Preiksa et al., 2005)	n=1204	position	4.6% (BW>2500g), 5.7% of all boys
UK, Cambridge Baby Growth Study	(Acerini et al., 2009)	n=742	position	5% (BW>2500g), 5.9% of all boys

*Does not seem to include high scrotal testis as cryptorchid testis

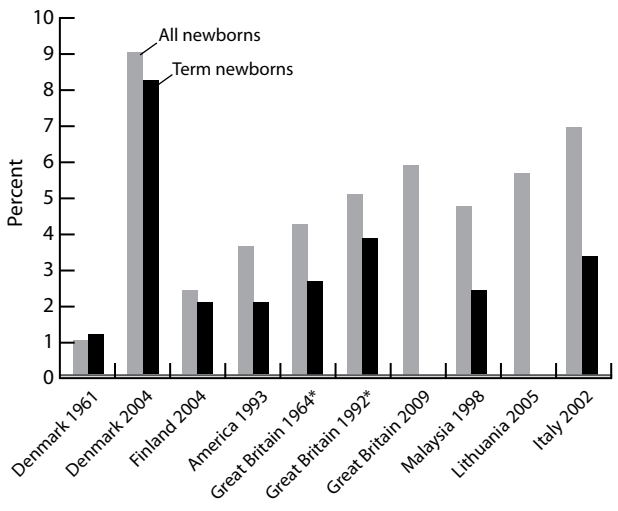

Figure 2.5. Increasing trends in cryptorchidism in newborn children in several European countries in various years.

reported in cryptorchid boys (Ferlin et al., 2003; Foresta et al., 2008), but rather these may be polymorphisms, because they were also frequently found in the normal population (El Houate et al., 2008; Nuti et al., 2008). In Finnish patients, no mutations either in *INSL3* or in *RXFP2* were found (Koskimies et al., 2000; Roh et al., 2003). However, down-regulation of these genes might lead to cryptorchidism. There are several other gene defects that cause cryptorchidism in gene-modified mice, e.g. *Hoxa10, Hoxa11* (Hsieh-Li et al., 1995; Rijli et al., 1995; Satokata, Benson & Maas, 1995; Overbeek et al., 2001; Daftary & Taylor, 2006), but there is hardly any evidence for their role in humans. Several syndromes include cryptorchidism as a part, and some of these are caused by known gene defects (Virtanen et al., 2007). Most often cryptorchidism, however, occurs as a single disorder. A single study reports lower cord blood levels of INSL3 in cryptorchid boys as compared to controls, suggesting that low INSL3 production may have affected testicular descent (Bay et al., 2007).

Laboratory evidence for EDCs causing cryptorchidism in rodent models of humans

In laboratory rodents, estrogens can down-regulate *INSL3* expression (Emmen et al., 2000; Nef, Shipman & Parada, 2000). Mice lacking INSL3 or its receptor (or with mutations in INSL3 or anti-Müllerian hormone) also exhibit bilateral intra-abdominal cryptorchidism with testes moving freely within the abdominal cavity. Over-expression of INSL3 or of

its receptor in females causes descent of the ovaries into the scrotal position. Prenatal exposure of laboratory rats or mice to 17-β estradiol or the non steroidal estrogen DES (see **Table 2.1**, section 2.1) disturbs the balance between androgens and estrogens and causes demasculinizing and feminizing effects in male embryos, including cryptorchidism. Moreover the estrogen receptor esr-1 (ERα) clearly plays a key role in the mechanism of this disorder, as mice lacking esr-1 but not esr-2 (ERβ) do not become cryptorchid when exposed to estrogens. Moreover, estrogen exposure down-regulates around 63 genes in the mouse fetal testes and up regulates 175, more than half of which are mediated by esr-1.

Epidemiological evidence that EDCs are linked to cryptorchidism in humans

There have been considerable efforts in the human health arena to link maternal exposure to particular chemicals, or groups of chemicals, with the incidence and severity of cryptorchidism. However, a fundamental problem with this approach is the difficulty in capturing exposures that have occurred during pregnancy. As such, the evidence from these studies is mixed: whilst the data linking cryptorchidism to EDC exposure in occupational settings are generally quite convincing (e.g. in agricultural workers; Pierik et al., 2004; Andersen et al., 2008; Weidner et al., 1998; Kristensen et al., 1997), studies that consider effects on the population in general are hampered by difficulties in deciding what chemical, or suite of chemicals, are of significance. All currently published studies are, however, employing a single chemical or group of chemicals approach. Studies that focus on the effects of each chemical in isolation appear to be less informative than those that deal with mixtures. For example, there is currently no evidence for an association between cryptorchidism and exposure to any of several organochlorine pesticides (Damgaard et al., 2006; Longnecker et al., 2002) or with concentrations of any of the individual congeners of polybrominated diphenyl ethers (PBDEs) found in mothers' milk (Main et al., 2007; Carmichael et al., 2010). When the sum of individual congeners was compared, however, Main et al. (2007) and Carmichael et al. (2010) reported significantly higher levels of PBDEs in the mothers' milk of boys with undescended testicles than in those with normal testicular development. Similarly, Damgaard et al. (2006) also detected a significant association between cryptorchidism and exposure to the eight most prevalent organochlorine pesticides. Moreover, analysis showed that the exposure of mothers had occurred long ago rather than recently (Shen et al., 2006). Fernandez et al. (2007) also found that the combined estrogenicity of placenta extracts was strongly related to rates of cryptorchidism. These observations echo the "something from nothing" phenomenon that has emerged from experimental studies with endocrine disruptor mixtures in which EDCs have been shown to act together in combination, even at low and individually ineffective concentrations (Silva, Rajapakse & Kortenkamp, 2002). Only a single study has identified differences in the levels of individual compounds between cryptorchid and normal boys, e.g. higher levels of heptachloroepoxide and hexachlorobenzene in fat samples of cryptorchid boys than in controls (Hosie et al., 2000). This was a small study and has not been repeated. Because the effects of EDCs on the same endpoint are expected to be additive, it would make more sense to use novel bioinformatics tools to integrate and analyze all exposures in order to elucidate whether distinct chemical signatures for different populations exist, e.g. in Denmark and Finland where rates of cryptorchidism differ (Krysiak-Baltyn et al., 2010).

Organochlorine pesticides and PBDEs are rather persistent, and some of them are anti-androgenic (Stoker et al., 2005). Although many of them have been banned after their initial introduction and by countries adhering to the Stockholm and Rotterdam Conventions (web sites www.pops.int and www.pic.int) they still persist in the environment as POPs and continue to add to the contaminant burden of the children (Darnerud et al., 2001; Betts, 2002; see Chapter 3.2). Apart from these well studied chemicals, phthalate plasticizer levels in mothers' urine have been associated with an anti-androgenic effect on the anogenital index of their sons (Swan et al., 2005), and with reduced testosterone and reduced sperm counts. Phthalate levels in breast milk were not, however, associated with the risk of cryptorchidism in these sons, but they were positively correlated with an increased luteinizing hormone/testosterone ratio, a sign of an anti-androgenic effect (Main et al., 2006b).

Several recent studies have addressed the question as to whether painkillers that inhibit prostaglandin synthesis contribute to the risk of developing cryptorchidisms: a study involving the Danish national birth cohort found an association between the use of paracetamol (an inhibitor of prostaglandin synthesis) in weeks 8-14 of pregnancy and a moderate increase in the occurrence of cryptorchidism (Jensen et al., 2010). For a second Danish cohort, Kristensen et al. (2011) reported that intake of paracetamol during the first and second trimester of pregnancy, and for longer than 2 weeks, increased the risk of giving birth to boys with cryptorchidism. The risk was even higher for mothers who had taken more than one compound, such as aspirin and ibuprofen, simultaneously. In the same paper, an association between the use of analgesics during pregnancy and cryptorchidism was not found for a Finnish cohort. Very recently, associations between paracetamol use during weeks 14 -22 of pregnancy and cryptorchidisms were also observed in a Dutch cohort (Snijder et al., 2012). In all these studies, drug use was established by questionnaire.

Low birth weight, being small for gestational age, prematurity and having other genital malformations are well-known risk factors for cryptorchidism (Hjertkvist, Damber & Borg, 1989; Group, 1992; Berkowitz et al., 1993; Berkowitz et al., 1995; Jones et al., 1998; Thong, Lim & Fatima, 1998; Akre et al., 1999; Weidner et al., 1998; Ghirri et al., 2002; Boisen et al., 2004; Preiksa et al., 2005). Mothers' smoking and alcohol consumption may also be risk factors, although studies on this are somewhat controversial. In a prospective cohort study, mothers' alcohol consumption was associated with an increased risk of cryptorchidism (Damgaard et al., 2007), whereas in registry- and interview-based studies including

severe cases, only binge drinking during early gestation was associated with a small increased risk (Jensen, Bonde & Olsen, 2007; Mongraw-Chaffin et al., 2008; Strandberg-Larsen et al., 2009). Many studies have not shown any association of mothers' smoking with cryptorchidism (Mongraw-Chaffin et al., 2008; Damgaard et al., 2008), whereas the use of nicotine substitutes was associated with an increased risk (Damgaard et al., 2008). However, heavy smoking was shown to be associated with an increased risk of bilateral cryptorchidism (Thorup, Cortes & Peterson, 2006), as was gestational diabetes (Virtanen et al., 2006).

2.3.2.3 Hypospadias

The condition in which the urethra opens on the ventral side of the penis or in the perineum instead of the tip is called hypospadias. It results from an incomplete closure of the urethral folds, leaving a split on the penis. (Källen et al., 1986; Kalfa et al., 2011). When the urethra opens to the glans or corona of the penis, it is called distal, whereas opening to the shaft or penoscrotal area defines hypospadias as proximal. Distal hypospadias is often left untreated and therefore is not registered in many malformation registries. Physiological phimosis may hide distal forms of hypospadias at birth, and these may become visible only later when the foreskin can be retracted behind the glans, which explains why the prevalence may differ when boys or men are evaluated at different ages. For example, in Denmark the birth rate of hypospadias was 1% and the cumulative incidence at 3 years was 4.6% due to appearance of distal hypospadias after loosening of the foreskin (Boisen et al., 2005).

Registry-based studies tend to underestimate rates of hypospadias (Toppari, Kaleva & Virtanen, 2001). This is caused by problems in clinical ascertainment, variable reporting to the registry, and differences of registry policies in recording distal cases. Many malformation registries do not register distal hypospadias, although these are most common in population-based clinical studies (Virtanen et al., 2001; Pierik et al., 2002; Boisen et al., 2005). The prevalence of hypospadias used to be between 0.4 and 2.4 per 1000 total births, but these estimates were recently found to be underestimates (Dolk et al., 2004) and there are clear time trends and regional variation (**Figure 2.6**). Increasing trends have been reported in several regions of Australia, Europe, and USA (Källen et al., 1986; Paulozzi, 1999; Toppari, Kaleva & Virtanen, 2001; Nassar, Bower & Barker, 2007; Lund et al., 2009).

Some of the controversies on trend analyses may have been influenced by the policy changes of many malformation registries that started to search for hypospadias more actively than previously, i.e. they did not wait for reporting by the clinicians but checked the diagnoses through the hospital discharge registries revealing many non-reported cases (Hemminki, Merilainen & Teperi, 1993). These changes have to be considered when assessing conflicting reports on the incidence data (Aho et al., 2000; Carmichael et al., 2003; Dolk et al., 2004; Porter et al., 2005; Fisch et al., 2009). Incidence data from prospective and cross-sectional clinical studies of hypospadias are presented in **Table 2.4**.

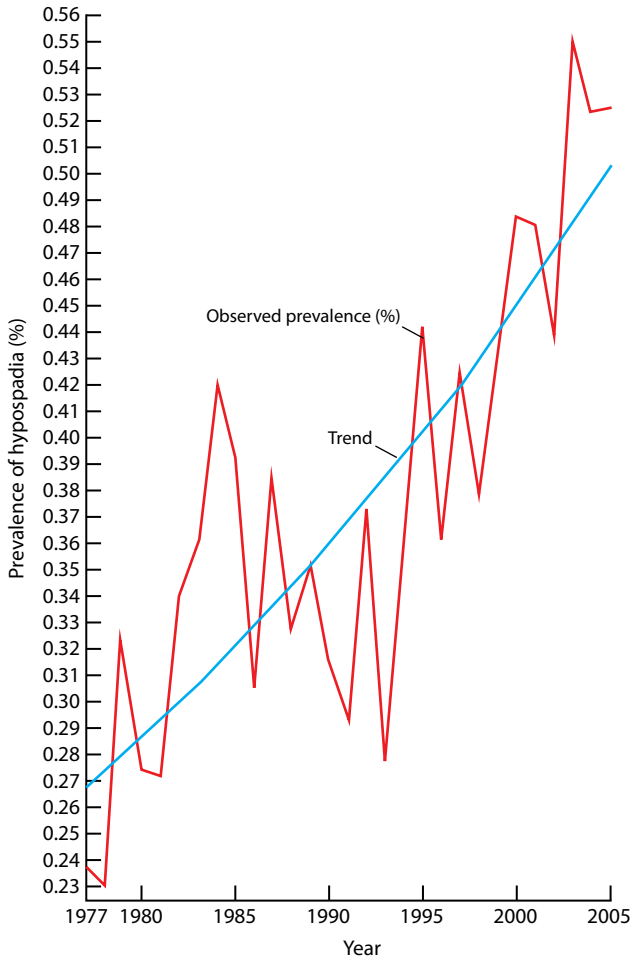

Figure 2.6. Observed prevalence of hypospadias among newborn Danish boys, 1977-2005 (Adopted from Lund et al., 2009).

Hormonal mechanism of hypospadias

Androgens regulate masculinization of external genitalia. Therefore any defects in androgen biosynthesis, metabolism or action during development can cause hypospadias. Gene defects causing disorders of testicular differentiation, conversion of testosterone to dihydrotestosterone or mutations in the androgen receptor can also result in hypospadias (Kalfa et al., 2008). In about 20% of patients with isolated hypospadias there are signs of endocrine abnormalities by the time of diagnosis (Rey et al., 2005).

Several genes that are involved in the penile development from the genital tubercle have been studied for their possible link to hypospadias, but few associations have been found (Kalfa et al., 2008; Wang & Baskin, 2008). The genes include *HOXA13, FGF 10,* and *FGF receptor 2* (Mortlock & Innis, 1997; Frisen et al., 2003; Beleza-Meireles et al., 2007). *Sonic Hedgehog* (*Shh*) is necessary for normal genital development in the mouse (Haraguchi et al., 2001; Perriton et al., 2002; Yucel et al., 2004), but no human mutations have been reported.

Table 2.4 Rate of hypospadias in boys in prospective or cross-sectional clinical (non-register based) studies (adopted from WHO, 2012).

Country	Reference	Study type	Rate of hypospadias
USA, Rochester, Minnesota, St. Mary's Hospital	(Harris & Steinberg, 1954)	Prospective study (n=4474)	0.70% (BW>2500g), 0.76% of all live-born boys
USA, ante partum clinic of the Sloane Hospital, New York City	(McIntosh et al., 1954)	prospective study on pregnant women and infants (n=2793 live-born males)	0.54% of live-born boys
USA, Collaborative perinatal project	(Myrianthopoulos & Chung, 1974)	prospective study (n=53394 consecutive single births (boys and girls))	0.80% of single-born boys (76% of cases detected at birth)
Korea, 38 hospitals	(Choi et al., 1989)	prospective study (n=7990)	0.21% of newborn boys
Southern Jordan	(al-Abbadi & Smadi, 2000)	Clinical study of 1748 boys (aged 6 to 12 years)	0.74% of boys
Finland, Turku, Turku University Hospital	(Virtanen et al., 2001)	Prospective cohort study (n=1505) Total hospital cohort (n=5798)	0.27% of live-born boys 0.33% of live-born boys
Netherlands, Rotterdam	(Pierik et al., 2002)	Prospective study (n=7292)	0.73% of newborn boys
Denmark, Copenhagen, Rigshospitalet	(Boisen et al., 2005)	Prospective cohort study (n=1072)	1.03% of live-born boys (at 3 years: 4.64% of boys (including also milder cases detected when physiological phimosis dissolved))
Bulgaria, 5 regions	(Kumanov et al., 2007)	Cross-sectional clinical study (n=6200 boys aged 0 to 19 years)	0.29% of boys

Activating transcription factor (ATF) 3 may also play a role on the basis of its expression levels locally in the foreskin (Liu et al., 2005). ATF3 is influenced by estrogens, suggesting that this could partly explain why estrogens increase the risk of hypospadias (Liu et al., 2006; Willingham & Baskin, 2007). Hypospadias is often a component in multi-organ syndromes.

Mutations in the gene *MAMLD1* (or *CXORF6*) lead to hypospadias (Fukami et al., 2006), but they are very rare (Ogata, Wada & Fukami, 2008; Ogata, Laporte & Fukami, 2009). The gene has a *NR5*/*SF1* target sequence and the defect affects androgen production (Fukami et al., 2008). Similarly, defects in *NR5*/*SF1* cause testicular dysgenesis (Bashamboo et al., 2010). This gene is one target for endocrine disruptors (Suzawa & Ingraham, 2008).

Genetic polymorphisms in androgen and estrogen receptors have been associated with the risk of hypospadias (Aschim et al., 2004b; Yoshida et al., 2005; Beleza-Meireles et al., 2006; Watanabe et al., 2007). Some of the studies have not been replicated successfully and further analyses in large populations are needed (van der Zanden et al., 2010, 2011; Wang et al., 2008).

Epidemiological evidence that EDCs cause hypospadias

Cryptorchidism and hypospadias have similar risk factors, e.g. being small-for-gestational age (Akre et al., 1999; Aschim et al., 2004a; Pierik et al., 2004; Akre et al., 2008). Estrogens and anti-androgens can cause both conditions, as evident in epidemiological studies following the children exposed to diethylstilbestrol (DES) during pregnancy (for review see Toppari et al., 1996). In addition, the sons of women who were exposed to DES in utero have a higher prevalence of hypospadias than other men, suggesting possible transgenerational effects via epigenetic mechanisms (Klip et al., 2002; Brouwers et al., 2006; Kalfa et al., 2008). All DES effects found in humans had been previously reported in experimental animals exposed to DES (McLachlan et al., 2001).

Association of exposure to pesticides with the risk of hypospadias has been analysed in several studies. A meta-analysis of nine studies showed an elevated, marginally significant risk associated with maternal occupational exposure, whereas paternal occupational exposure was not statistically significant (Rocheleau, Romitti & Dennis, 2009). In the Avon Longitudinal Study of Parents and Children (ALSPAC; a large British child cohort), vegetarian diets of mothers were associated with an increased risk of hypospadias (North & Golding, 2000), while a Swedish study showed a decreased risk for sons of mothers who had fish or meat in their diet during pregnancy (Akre et al., 2008). Whether vegetarians were exposed to more pesticides than omnivorous women is not known. Sub fertility and the use of assisted reproductive techniques increase the risk of hypospadias (Sweet et al., 1974; Czeizel 1985; Wennerholm et al., 2000; Klemetti et al., 2005; Källen et al., 2005). The risks posed by pharmaceutical sex steroids other than DES are controversial. In the past, the use of progestins was associated with an increased risk of hypospadias (Czeizel, Toth & Erodi, 1979; Calzolari et al., 1986), but a more recent meta-analysis of fourteen studies did not find any association between exposure to sex steroids (excluding DES) during the first trimester and external genital malformations (Raman-Wilms et al., 1995). Progestins have been recently introduced again to the market in USA as preventive medicines against threatening miscarriage without evidence of efficacy or lack of untoward effects to date (Wahabi et al., 2011).

2.3.2.4 Reduced semen quality

A meta-analysis from 1992, with results from 14,947 men, included in a total of 61 papers published between 1938 and 1991, indicated that there had been a decline in semen quality

during a period of half a century (Carlsen et al., 1992). The paper was followed up by several studies in which people used available databases in search for trends. Although the results caused controversy (Jouannet et al., 2001), a new meta-analysis with expansion of the data to 101 studies gave similar results (Swan et al., 2000). However, the question of declining sperm counts continues to cause controversy, even today (Jouannet et al., 2001; Skakkebæk et al., 2011). The reason for the controversy may partly be explained by geographical differences in semen quality (Jørgensen et al., 2001), and partly by differences in methods for semen analysis and variation in results within individuals (Jørgensen et al., 1997). Furthermore, all the studies on which the meta-analyses were based were retrospective. To test the hypothesis that semen quality might have deteriorated, Nordic, Baltic, German, Spanish and Japanese investigators have since carried out prospective studies on men from the general population (Jørgensen et al., 2011; 2013). The common finding has been that a significant proportion of young men have semen quality below what is considered to be compatible with good fecundity, although geographical variation exists between countries and between different parts of the same country. Several studies have shown that a sperm concentration below 40 mill/mL is associated with reduced fecundity (Bonde et al., 1998; Guzick et al., 2001; Skakkebæk et al., 2010). 20-40% of young men have a sperm concentration below this level. The problems with human semen quality were recently confirmed in a large French study; decline in semen concentration and morphology in a sample of 26 609 men close to general population between 1989 and 2005 in France (Rolland et al., 2012).

Since 1980, WHO has published five editions of guidelines for semen analysis (WHO, 1980; 1987; 1992; 1999; 2010). The guidelines have been of great value for world wide standardisation of the analysis. WHO has also included reference ranges for semen. In the most recent edition of the guidelines, the reference ranges were changed to reflect the distribution of men who have sired children. The lower cut-off level was estimated to be 14 mill/mL (WHO, 2010). However, as some authors have suggested that no clear borderline exists between the sperm counts of sterile and fertile men, caution should be taken to categorize all men with sperm counts above 15 mill/mL as normally fertile (Skakkebæk et al., 2010; Björndahl, 2011).

It is well known from animal studies that males can sire normal numbers of children in spite of severe defects in spermatogenesis. However, at some tipping point impaired spermatogenesis will result in lower fertility (Andersson et al., 2008; Slama et al., 2002). The question is whether some human populations have reached that point. Besides the low number of sperms in a proportion of men, the average young Scandinavian man has more than 90% abnormal spermatozoa (Jørgensen et al., 2006).

It is still crucial to know whether the increasing use of assisted reproductive techniques (Nyboe Andersen & Erb, 2006) and widespread low fertility rates seen in many industrialized countries (Hvistendahl, 2011) are caused by social factors alone or are also related to male sub fertility (Jensen et al., 2008). Importantly, low sperm counts may be related to poor Leydig cell function (Andersson et al., 2004) and, in some cases, they may be a symptom of TDS (Skakkebæk, Rajpert-De Meyts & Main, 2001). Poor semen quality is clearly also linked to TGC (Petersen et al., 1999) and undescended testis and some cases of hypospadias (Giwercman & Giwercman, 2011). However, most cases of poor semen quality in infertile men are not linked to cryptorchidism and hypospadias. In spite of this, recent evidence from studies of anogenital distance (AGD) in men indicate that poorer semen quality was associated with a shorter AGD, indicating that the low sperm count in some cases could have a prenatal origin, *even* if it is not accompanied by undescended testis and/or hypospadias (Mendiola et al., 2011; Eisenberg et al., 2011).

Evidence of a role of endocrine disruptors in causing low semen quality

Although genetic factors play important roles in causing poor semen quality in some men (Krausz, 2011), most cases of poor semen quality have no known etiology. Smoking and particularly exposure to maternal smoking in utero have been associated with reduced sperm counts (Jensen et al., 2004; Ramlau-Hansen et al., 2007; Ravnborg et al., 2011). A role of EDCs has been hypothesized, but to date there are no clear data except for some rare cases of environmental or occupational accidents where men were exposed to toxic agents like DBCP, which caused azoospermia in workers producing or using the pesticide (Whorton et al., 1979), or dioxin (Mocarelli et al., 2011), which reduced semen quality. Interestingly, in both the DBCP and dioxin cases, the sex ratio was skewed towards an excess of girls fathered by those men during recovery from the exposure. Ongoing preliminary studies in many countries are focussing on possible effects of POPs (Meeker & Hauser, 2010), PFA (Joensen et al., 2009), and non-persistent chemicals (including phthalates (Hauser, 2008), bisphenol A (Mendiola et al., 2010, Li et al., 2011) and DDT/DDE (reviewed in IPCS, 2011) on reproductive functions. The work to unravel the possible effects is cumbersome, as some effects may be prenatal (as the AGD studies suggest) and other effects may be postnatal or perhaps most likely a combination of several types of effects. Considering that the total exposome covers the whole life time and perhaps hundreds of exposures in varying concentrations, links to specific chemicals – if they exist - may be very difficult to establish.

Whilst epidemiological studies of male genital malformations have correlated effects with exposures occurring in utero, studies on semen quality have, almost exclusively, focused on the influence of exposures experienced in adulthood. One of the few exceptions involved an investigation into the consequences of PCB exposure in fetal life for semen quality in later life and was conducted among victims of the Yuscheng incident in Taiwan. Between 1978 and 1979, large quantities of cooking oil contaminated with PCBs were consumed by the Taiwanese people. Guo et al. (2000) examined semen quality among boys whose mothers consumed the oil during pregnancy and found that the boys exposed in utero had sperm with abnormal morphology and reduced motility. Similar effects were observed in men who consumed the cooking oil in adulthood

(Hsu et al., 2003). These men had higher numbers of sperm with abnormal morphology than unexposed men. However, other determinants of semen quality were similar between the two groups. In contrast, the comparison of blood PCB levels in men with poor and normal semen quality did not differ significantly, although an inverse relationship between sperm counts and PCB levels was found among men with normal semen quality (Dallinga et al., 2002).

2.3.2.5 Decreased testosterone

There are two population-based studies indicating a decline of testosterone levels in a birth-cohort dependent manner, i.e. younger generations have lower testosterone levels than the older ones at the same age (Andersson et al., 2007; Travison et al., 2007). Obesity contributes to a decreased testosterone concentration, but it does not explain the adverse trends observed in the above-mentioned studies. Similar findings have been repeated also in Finland (Perheentupa et al., in press). No exposure associations are available.

2.3.2.6 Testicular dysgenesis syndrome

As already stated, hypospadias, cryptorchidism and TGC may, in fact, be related components of a single underlying condition, termed 'testicular dysgenesis syndrome' (TDS), originating during fetal development. Consequently, evidence to support chemical causation of any one of these disorders also adds credence to the hypothesis of chemical causation of the associated disorders. TGC is associated with other reproductive disorders such as cryptorchidism, lower testosterone levels and intersex conditions with hypospadias, in line with a hypothesis of a common origin of these testicular problems (Skakkebæk et al., 2007; Møller & Skakkebæk, 1999). In Finland a recent increasing trend in TGC has coincided with a declining trend in semen quality (Jørgensen et al., 2011), whereas the increase in Denmark seems to have leveled off, at least with regard to TGC (Schmiedel et al., 2010)

Cryptorchidism is a risk factor for testicular cancer. Men with a history of cryptorchidism have a 4-6 fold higher risk of developing testicular cancer than men without cryptorchidism (Dieckmann & Pichlmeier, 2004; Schnack et al., 2010a). However, only about ten percent of men with testicular cancer have been cryptorchid. Bringing the cryptorchid testis down to the scrotum (orchidopexy) does not affect the cancer risk much. Thus, these two disorders share etiological factors rather than having a direct causal relationship with each other, i.e. cryptorchidism is not the cause of testicular cancer. Due to shared etiological factors, a high incidence of cryptorchidism is accompanied with a high rate of testicular cancer, which is apparent in Denmark and Finland; these countries have high and low incidence rates, respectively (Boisen et al., 2004; Jacobsen et al., 2006). Therefore any causal relationship of cryptorchidism with environmental effects can also be considered a putative risk factor for testicular cancer.

Semen quality and fertility are closely related to cryptorchidism (Lee & Coughlin 2001; Virtanen et al., 2007). This connection is reflected also by the differences in semen quality between Finland and Denmark. Sperm counts are significantly lower in Denmark than in Finland (Jørgensen et al., 2001; Jørgensen et al., 2002). The Danish-Finnish cohort study of cryptorchidism, where the testes were measured by ultrasound and reproductive hormones were analysed at the age of three months, showed differences in genital size in early childhood (Boisen et al., 2004; Main et al., 2006a). In Denmark, the testes were smaller and grew slower than in Finland (Main et al., 2006a). Inhibin B levels, reflecting testicular volume, were also lower in Danish boys than in Finnish boys.

The incidence of hypospadias is much lower than that of cryptorchidism, but these disorders are also linked (Toppari, Kaleva & Virtanen, 2001; Schnack et al., 2010b). The incidence of hypospadias differs between Denmark and Finland similar to testicular cancer and cryptorchidism (Suomi et al., 2006; Virtanen et al., 2001; Boisen et al., 2005). All these disorders and spermatogenic problems are linked to androgen action and hormonal regulation during development (Sharpe & Skakkebaek, 2008).

Laboratory evidence for EDCs causing testis dysgenesis syndrome in rodent models of humans

Much of the laboratory-based research into the reproductive implications of EDC exposure in men has been carried out using the rat as a model. This is because, whilst male rats differ from men to some extent with regard to steroidogenesis (discussed by Scott, Mason & Sharpe, 2009), in general the processes underlying their testicular development are thought to be remarkably similar. The assessment of reproductive toxicity in rats comprises a test in which chemicals are administered to dams during gestation. This method has proven to be extremely informative, not only in helping to identify chemicals that interfere with male reproductive development, but also in aiding the discovery of the male programming window and in demonstrating the irreversible nature of the ensuing events. The discovery of the male programming window has been of particular significance in helping to identify the reproductive toxicity associated with certain phthalates and other types of EDCs that only elicit an effect if dosing occurs during a particular window of gestation. Moreover, the analysis of the male offspring produced by these studies has demonstrated that all of the constituent elements of TDS can be recapitulated in the rat, as in men, with the exception of TGC. Male rats exposed to certain phthalates and other chemicals that block the actions of androgen exhibit a range of symptoms, including non-descent of testes, malformations of the external genitalia (similar to hypospadias), poor semen quality and malformations of other sex organs (Foster, 2005; 2006). Evidence of the induction of this so-called "phthalate syndrome" in rats, which closely mirrors TDS in men, suggests that EDCs may be involved in the causation of male reproductive dysgenesis in the human population.

Mechanistically, phthalate syndrome appears to result from lowered fetal testosterone and malformations of the internal genitalia as a consequence. As the development of the male

reproductive tract, prostate and external genitalia also depends on dihydrotestosterone (DHT), a more potent androgen derived from testosterone, lower testosterone concentrations can also cause malformations such as hypospadias. Male rats also require DHT for the regression of nipple anlagen and for the growth of the perineum to produce the normal male AGD. Reduced DHT levels in the wake of suppressed testosterone synthesis also leads to retained nipples and feminized AGDs. These additional signs are useful endpoints for laboratory studies and, as such, the rodent model has been widely used to explore a range of pertinent issues in the EDC field. The data generated have been crucial, for example, in contributing to the controversy regarding bisphenol A and its effects at low doses (see Vanderberg et al., 2012), demonstrating the capacity for mixtures of antiandrogens to act in combination (Rider et al., 2008), and aiding in the identification of new and emerging EDCs, such as inhibitors of prostaglandin synthesis (Kristensen et al., 2010).

There is a growing body of experimental evidence showing that, apart from phthalates, other chemicals that inhibit androgen production or action (anti-androgens) can disturb testicular descent (e.g. Hotchkiss et al., 2010; Rider et al., 2010), and cause hypospadias (e.g. Wilson et al., 2008, but see also **Table 2.1** and section 2.3.5), lowered testosterone and decreased sperm counts. The central role of androgens in both penile development and testicular descent is an important physiological link between cryptorchidism and hypospadias, suggesting a common etiology for these conditions.

Several chemicals can act as androgen receptor antagonists in rodents, and this mode of action is easy to assess also in vitro and by using QSAR methods. The fungicide procymidone and the insecticide metabolite p,p´-DDE are examples of such anti-androgens acting at the receptor level (Wilson et al., 2008). Vinclozolin is not an anti-androgen by itself, but its two metabolites are androgen receptor (AR) antagonists (Kelce et al., 1995). Diethylhexyl (DEHP) phthalate, benzyl butyl phthalate and dibutyl phthalate are compounds that exert anti-androgenic action by inhibition of testosterone production, without affecting AR (Wilson et al., 2008). Some chemicals have both of these anti-androgenic properties, i.e. they inhibit testosterone synthesis and block its action on the receptor. The herbicide linuron and fungicide prochloraz are examples of this (Wilson et al., 2008). When any of these compounds are combined in mixtures, they show dose-additive effects rendering adverse effects even when each of them is in the mixture below its individual NOAEL (Rider et al., 2008; Christiansen et al., 2008).

Anogenital distance (AGD)

The effects of EDCs reported in the rats and mice have also been used to inform human epidemiology studies. In this respect, AGD in baby boys, as in rodents, is a valuable biological marker of disruption of androgen action in fetal life and is inversely related to the risk of cryptorchidism and hypospadias in baby boys (Swan et al., 2005). The study by Swan et al. (2005) investigated changes in AGD relative to maternal levels of urinary phthalate metabolites and found significant relationships between the highest levels of maternal phthalates and shortened (i.e. feminised) AGD in young boys. The patterns reported mirror experimental evidence from rats maintained under controlled laboratory conditions and provide strong evidence that developmental phthalate exposures contribute to disruptions of androgen action in the human population. An expansion of the Swan study, which incorporated a larger number of mother-infant pairs, has confirmed the earlier results (Swan, 2008) and correlations between AGD in boys and maternal exposure to phthalates were also reported in a study of Mexican women (Bustamante-Montes et al., 2008). Moreover, reduced AGD was also found in adult men with corrected hypospadias or cryptorchidism (Hsieh et al., 2008). Poor fertility and impaired semen quality have also been associated with short AGD (Eisenberg et al. 2011; Mendiola et al., 2011).

Although the processes of steroidogenesis and hormone action are essentially the same across most mammalian species, studies suggest that the detailed regulation of testosterone synthesis and pathways through which chemicals act obviously show species differences that will be important for risk assessment of some chemicals. With phthalates, for example, when the rat model of in utero phthalate exposure was extended to the mouse, no suppression of testicular testosterone or its biosynthetic genes was observed (Gaido et al., 2007; Johnson et al., 2011), thus raising the question of whether human health effects of phthalates are better predicted by the rat or the mouse? Moving closer to humans, monobutyl phthalate (MBP) was shown to suppress testosterone levels acutely in the neonatal marmoset, although this suppression was rapidly compensated for, presumably via elevation of luteinizing hormone (LH) levels. In human cells, studies using fetal human testis explants failed to find any effect of MBP on testosterone production (Hallmark et al., 2007; Lambrot et al., 2008) and human fetal testis xenografts exposed to DBP showed no decrease in expression of the steroidogenic genes responsible for fetal testosterone biosynthesis or a reduction in testosterone either (Mitchell et al., 2012; Heger et al., 2012).

Heger et al. (2012), however, found that multinucleated germ cells were induced by phthalates in all three species, human, mouse and rat, showing clearly that concordance across species can be present for some responses but absent for others. Multinucleated spermatogonia have been observed in the testes of both juvenile cryptorchid boys (Cortes et al., 2003) and adult men (Nistal et al., 2006) presenting with carcinoma in situ of the testis. The long-term effects of these dysgenetic germ cells remain unclear.

Other factors may also play critical roles in TDS. Very recently, van den Drische et al. (2012) showed a potentially important role for Chicken Ovalbumin Upstream Promoter-Transcription Factor II (COUP-TFII) in Leydig cell (LC) steroidogenesis that may partly explain phthalate syndrome seen in rats. Exposure of fetuses to DBP dose-dependently prevented the age-related decrease in Leydig cell COUP-TFII expression and the normal increases in Leydig cell size and intratesticular testosterone, thus revealing a further aspect to the mechanisms through which phthalates may act which may or may not be preserved in humans.

The answer to the question of whether phthalates affect steroidogenesis in humans as they do in the rat may mean that concerns about current exposures to phthalates are redundant and that we should focus on chemicals that act via other mechanisms, such as anti-androgenic pesticides and fungicides. Regardless of the conclusion reached, it seems likely that a risk assessment for TDS will have to take into account coexposures to environmental chemicals that are "antiandrogenic" via one or more mechanisms.

2.3.3 Evidence for endocrine disruption of the male reproductive system in wildlife

2.3.3.1 Wild mammals

Some wildlife populations appear to exhibit a suite of demasculinizing and/or feminizing disorders consistent with the symptoms seen in human populations, indicating that the human and wildlife evidence should be considered in parallel when assessing whether EDCs contribute to the etiology of male reproductive disorders. In addition, there is a wealth of data from other types of animal studies that may also be considered as evidence. For example, cryptorchidism has been reported across many farmed and domestic animals, including horses, pigs, rams, rabbits and cattle, as well as cats and dogs (e.g. it was reported with a frequency of up to 6.6% in the case of a sample of more than 300 stray dogs sampled in the tropics; Ortega-Pacheco, 2006). These studies may present opportunities to learn more about its etiology and also serve to highlight the similarities between effects occurring in humans and in animal species. Indeed, a recent study by Bellingham et al. (2012) showed that exposure of sheep to chemicals associated with sewage sludge amended pastures showed that 5 of 12 sludge-exposed rams exhibited major spermatogenic abnormalities, including major reductions in germ cell numbers per testis or per Sertoli cell and more Sertoli cell-only tubules, when compared with controls, which did not show any such changes. Hormone profiles and liver concentrations of a suite of chemicals were not measurably affected by exposure. Such effects seen in real-world exposures could have adverse consequences for sperm counts and fertility in some of the exposed males.

The Florida panther attracted attention when reports indicated a sharp rise in the incidence of cryptorchidism over a thirty year period, beginning in the early 1970s. By 2001, this meant that over half of the population was cryptorchid (mostly unilaterally) and, in addition, almost a quarter of the juveniles exhibited signs of delayed testicular descent (Buergelt, 2002; Mansfield & Land, 2002; Buerguelt, Homer & Spalding, 2002). Coincident with cryptorchidism, these individuals also exhibited reduced testicular volume, low sperm motility and density and semen volume, as well as higher numbers of morphologically abnormal sperm (flaws in the acrosome and mitochondrial sheaths) in comparison with other populations with much lower levels of cryptorchidism. Although this panther population is known to be severely inbred due to its small size, an analysis by Facemire, Gross & Guillette (1995) suggested that genetic composition does not fully explain the observed reproductive abnormalities and proposed dietary exposure to EDCs as a causal factor in the etiology of this apparent male reproductive syndrome. This was refuted by Mansfield & Land (2002), who reported drastically reduced cryptorchidism rates in the progeny of the Florida panther during a genetic restoration plan in which eight female puma from Texas were released into the Florida panther population in 1995. None of the progeny resulting from genetic restoration efforts have been cryptorchid, thus suggesting that cryptorchidism in panthers is genetically rather than environmentally based.

Both uni- and bilateral cryptorchidism, along with many of the other symptoms associated with TDS in humans, have been reported in large numbers (68% in some populations) of Sitka black-tailed deer on Kodiak Island, Alaska. Bubenik & Jacobson (2002) obtained cryptorchid testes from affected individuals and found that these contained malformed or degenerated seminiferous tubules, which contained Sertoli cells but lacked spermatogenic activity. Other abnormalities were evident, such as carcinoma in situ-like cells (possible precursors of seminoma) and microlithiasis, a condition also observed in men with TDS (Skakkebæk, 2004). In addition to these signs of testicular dysgenesis, the affected population also suffered from abnormal antler development. Although genetic causes cannot be ruled out completely, a recent critical analysis favours exposure to environmental contaminants as a likely causal factor (Latch et al., 2008). Such exposure may transform the spermatogenic cells, affect the development of primordial antler pedicles, and also block transabdominal descent of fetal testes, thereby resulting in testis-antler dysgenesis in the affected population (Veeramachaneni, Amann & Jacobson, 2006; **Figure 2.7**).

Similar abnormalities including undersized and mispositioned scrota and ectopic testes were also reported in 67% of 254 male white-tailed deer sampled in the Bitterroot Valley of west-central Montana, USA, between 1996-2000 (Hoy et al., 2002). A high incidence of cryptorchidism in wildlife populations could contribute to population declines and low genetic diversity. In the Sitka deer, however, despite a low proportion of potentially fertile male (only 32% in one population of the Sitka-tailed black deer in Alaska), population growth rates and levels of neutral genetic diversity remain high due to a reproductive strategy whereby few males impregnate many females.

Male polar bears have also shown a multitude of reproductive disorders in recent years that may be linked to their accumulation of high concentrations of persistent organochlorine pollutants such as PCBs (see Chapter 3.2.1). In this respect, the analysis of the reproductive organs of 55 male bears from East Greenland by Sonne et al. (2006) revealed negative correlations between testes size and baculum (penis bone) length and weight in relation to their tissue concentrations of organohalogen pollutants. There was also evidence of testicular fibrosis, atrophy, and inflammation in 20 of the bears analysed. These symptoms may pose a threat to

the reproductive capacity of contaminated populations via their effects on sperm quality and quantity and by compromising their ability to perform coitus, particularly in view of the fact that females of this species are induced ovulators. However, there is no evidence for this as yet. In addition, an inverse relationship between organochlorine contaminant exposure and blood testosterone levels has been reported in polar bears from the Svalbard region (Norway), in which body burdens of organochlorines are particularly high (Oskam et al., 2003). Low fertility and rates of reproduction have also been reported for this population relative to others (Derocher et al., 2003), although whether this is linked to chemical exposure or to differing population age structure and harvesting pressure is the source of some debate (Haave et al., 2003).

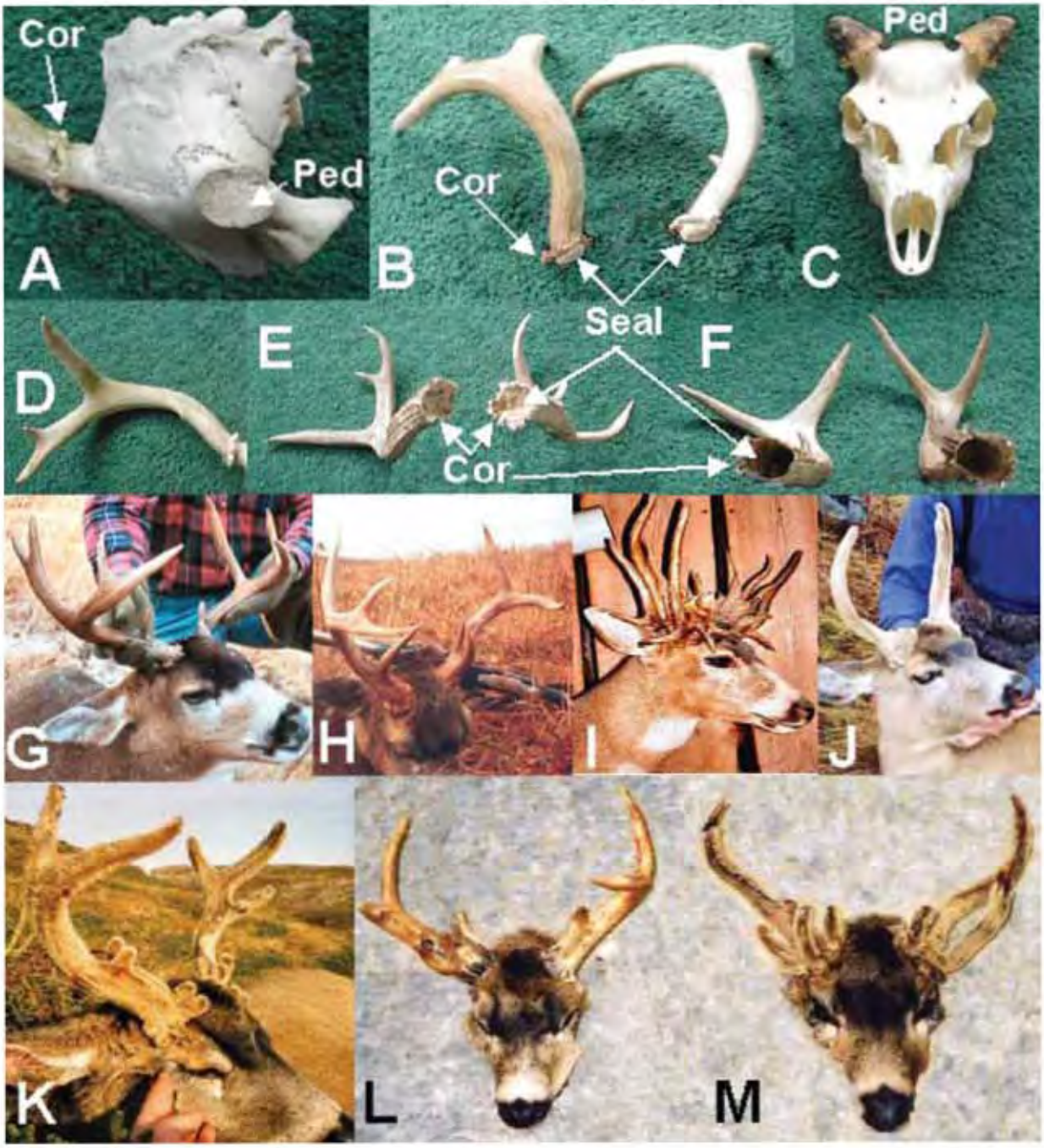

Figure 2.7. Antler dysgenesis in Sitka black-tailed deer. A: Normal skull with coronet (Cor) and concave antler pedicle (Ped) designated. B: Pair of normal antlers each with a convex seal designated. C: Skull with atypical and extremely abnormal, convex antler pedicles (Ped) from a BCO deer. D: Normal antler partially shown in A. E: Pair of antlers each with a thickened base and concave seal (compare with B). F:Pair of antlers each with a thickened base, including extra points, and an extremely concave seal; associated with skull in C. G: Normal antlers. H: Abnormal antlers, typifying those classified as "polished with sharp tips" (compare with G). Also compare tips in B, D, and G (normal) with those in E, F, and H (polished with sharp tips). I through M are abnormal antlers. I: Extraordinary antlers of deer shot in 1967 by N Sutliff. J: Deer with spike antlers, despite age and size, still in velvet and arising from a thick base. K: Deer with unusual antlers bearing extra "points" or " nubs" still covered with velvet. L: Deer with odd antlers and retaining some velvet. M: Deer with bizarre antlers still covered with velvet. From Veeramachaneni, Amann & Jacobson, 2006, supplemental material (Figure used with publisher's permission).

Despite the examples outlined above, which are well known and widely studied, there are a lower number of documented examples of reproductive dysfunction in wild male mammals than one would expect. It is unclear whether this is because the phenomenon is relatively rare, or whether this paucity of data is simply a reflection of the lack of studies that have been done. The paucity of literature suggests that further studies of male reproductive system disorders in mammalian wildlife are needed and that they should focus on predators, which are expected to have a greater EDC burden (as a consequence of biomagnification; see Chapter 3.2.1). In this respect, particularly high concentrations of hydrophobic contaminants (PCBs and brominated flame retardants) have been measured in marine mammals, which, with the exception of plankton-feeding whales, are top predators (see Aguilar et al., 2002; Hansen et al., 2004; Lie et al., 2004; Ross et al., 2000; Noel et al., 2009). Such mammals may be more plastic in terms of their sexual development than humans, potentially making them more sensitive to the effects of EDCs. For example, with regard to cetaceans, whilst both pseudo and true hermaphroditism have been reported (with the external phenotype appearing to be female, but with the internal reproductive organs, or elements thereof, appearing male), there is also evidence of more subtle abnormalities, such as the development of ovotestes (Murphy et al., 2010). This indicates that the suite of symptoms that reflect testicular dysgenesis may be greater in some mammalian species than in others, and that their sensitivities to EDCs may vary. Although it is very difficult to establish causation in such cases, the possible implications of these isolated observations for these species can also not be ignored.

2.3.3.2 Non-mammalian vertebrates

Examples of male reproductive system disorders, occurring concomitantly with chemical exposure, can be provided for all non-mammalian vertebrate classes. Perhaps one of the best documented cases is that of a reptile exposed to high concentrations of pesticides (dicofol, DDD, DDE and DDT) following an accidental spill into a tributary of Lake Apopka in 1980. This had a profound effect on the resident American alligator population. Alongside a dramatic decline in juvenile recruitment, Guillette and colleagues reported various malformations of the male genitalia, including reduced phallus size and histological changes in structure of the testes, along with depressed plasma testosterone concentrations (reviewed by Guillette et al., 2000). However, subsequent studies of alligators from other contaminated lakes yielded inconsistent findings: plasma testosterone concentrations were not reduced at contaminated sites relative to those measured at a control site (Milnes et al., 2002; Gunderson et al., 2004). Furthermore, there was no evidence of a correlation between plasma testosterone, phallus size and contamination status, though a correlation was anticipated based on the Lake Apopka data (Gunderson et al., 2004). The reason for this lack of consistency cannot be ascertained using currently available data. However, it is clear that contaminant exposure can profoundly affect the reproductive development of male alligators, potentially compromising their reproductive capabilities, and that reptiles can exhibit a suite of symptoms in response to chemical exposure which are not dissimilar to those reported in humans and other mammalian species.

Reproductive endocrine disruption has also been reported across a range of bird species since the 1950s. Historically, investigations centred on the issue of eggshell thinning in predatory birds in relation to organochlorine exposure, which ultimately prompted the ban on the use of DDT in North America and Europe. This led to a reduction in body burdens in birds and an improvement in eggshell thickness, with the subsequent recovery of the affected populations (reviewed by Cheek, 2006). However, various other abnormalities have been reported, some of which appear to be consistent with the feminization and/or demasculinization of the male reproductive system. In this respect, the retention of ovarian tissue in the testes of male terns was reported in a breeding colony on Bird Island, Massachusetts (USA) and the prevalence (although not the severity) of this abnormality was associated with PCB and dioxin levels in the developing eggs (Hart et al., 1998). Approximately half of the newly-hatched male chicks had primordial germ cells, which were arranged in a female-like pattern, but no oviducts. A subsequent study of the same colony, however, revealed that these intersex characteristics were no longer apparent by the time the chicks were 21 days old and thus were considered unlikely to influence fertility (Hart et al., 2003). The capacity for EDCs to cause testicular dysgenesis in avian species is supported by laboratory evidence of the effects of chemical exposure on testicular structure and size, seminiferous tubule diameter, delayed germ cell differentiation and sperm quantity (reviewed in Edwards, Moore & Guilette, 2006). In addition, there is evidence of a relationship between high EDC burden and lowered testosterone levels in the eggs of wild birds (Arctic-breeding glaucous gulls; Verboven et al., 2008). Contaminant-induced changes in the ratios of estrogen to testosterone were also reported, indicating that testicular dysgenesis in birds has a similar aetiology to that reported in other vertebrate classes.

Male amphibians also appear to be vulnerable to EDC exposure; intersex has been reported in a range of Anuran species (i.e. frogs and toads) exposed to contaminants in the field. In this respect, Reeder et al. (2005) used historical specimens of the cricket frog, which were collected from 1830-1996, to analyse both temporal and spatial trends in the rates of intersex across the state of Illinois, USA. The authors found that that incidence was low pre-1930 (1.2% from 1852-1929), increased during the period of industrial growth (7.5% from 1930-1945), and was highest during the period of industrialisation and use of organochlorines (11.1% from 1946-1959). It then decreased when sales of DDT were restricted (6.3% from 1960-1979) and continued to decrease in more recent years (2.7% from 1980-1996). Over the total period, the incidence was highest in industrialised areas (10.9%), intermediate in agricultural areas (4.9%) and lowest in less intensively-managed areas (2.6%). Further evidence that testicular maldevelopment is

linked to EDC exposure is provided by a study on leopard frogs by Hayes et al. (2003), which revealed that, out of eight test sites, the only site that had no detectable atrazine was also the only site at which there were no intersex frogs. In addition, gonadal dysgenesis (underdeveloped testes with poorly structured, closed lobules and low to absent germ cells) were observed in frogs at one of the sites that had relatively high levels of atrazine. However, robust evidence that there was a relationship between the rate of reproductive abnormalities and atrazine exposure was lacking. This was also the case in a subsequent study involving leopard frogs, in which the incidence of intersex did not correlate with atrazine exposure on its own; however, there was a positive association between intersex incidence and the total concentrations of all analysed pesticides (McDaniel et al., 2008). Reduced testosterone levels in males from agricultural sites were also reported, a finding that was mirrored in a field study of cane toads, which revealed that intersex correlated with agricultural land use and that intersex toads had lower levels of testosterone (McCoy et al., 2008). Further signs of feminization, including changes in colour, forelimb size and number of nuptial pads, were also reported in toads at the affected sites. Thus, although research into the effects of EDCs on amphibians has been hampered by a range of factors (e.g. low sample size, lack of data on chemical exposures), and the issue concerning atrazine, in particular, remains contentious, it would appear that endocrine disrupting effects on amphibians are manifested as a suite of symptoms, which may be similar to testicular dysgenesis syndrome seen in men.

The feminization of male fish living near to the outfalls from sewage treatment works (STW) plants is consistent across investigators, geographical regions, species and habitats (Cheek, 2006). Various conditions have been reported in wild caught male fish, including the abnormal induction of egg yolk protein (vitellogenin; VTG), as well as a range of abnormalities of the reproductive system, such as altered spermatogenesis, intersex and the feminization of ducts. There have been considerable efforts to determine the functional significance of these abnormalities. In this respect, studies by Jobling and colleagues on roach inhabiting UK water courses have been most informative in terms of linking intersex and vitellogenin (egg yolk protein) induction in male fish to their reproductive capability. For example, Jobling et al. (2002b) reported that all of the phenotypically male fish from polluted sites were intersex and contained female-like ducts and, in addition, exhibited delayed spermatogenesis compared to either intersex or normal fish from reference populations. Intersex roach from polluted sites also had a reduced percentage of spermiating males and lower milt volume, though sperm density was adversely affected at only one polluted site (the Aire), as well as altered testosterone levels compared to normal males. In a related study by Jobling et al. (2002b), intersex fish were again observed to have a lower percentage of spermiating individuals, although milt volume was not affected. Sperm motility and velocity were also low and these characteristics correlated with the severity of intersex. Furthermore, the fertilisation rates of intersex fish from polluted sites was reduced from 93 to 68%, although this difference was only observed in one out of two of the years studied. More recently, competitive breeding experiments with wild roach revealed that reproductive performance was negatively correlated with the degree of intersex, with a 76% reduction in the number of offspring parented by the most feminized individuals (Harris et al., 2011). In some species of fish, evidence of feminization of male urogenital papillae, a condition denoted as morphologically intermediate papilla syndrome (MIPS), has also been seen in wild populations and was more prevalent at sites contaminated with estrogens.

Although most fish species do not have sex chromosomes, they do share other sex differentiation gene products with humans and other mammals (such as DAX-1, DMRT-1, cytochrome P450 ovarian form, cyp19a1, etc.) that have been found to change according to the sexual phenotype of the developing gonads. In the genetically male medaka fish, for example, exposure of male embryos to exogenous estrogen during the process of sex differentiation up-regulates ESR-1, inhibits the expression of enzymes involved in androgen biosynthesis (CYP17, 11 beta-HSD and 17β-hydroxysteroid dehydrogenase [17 beta-HSD]) and testis differentiation (anti-Müllerian hormone [AMH] and doublesex and mab-3 related transcription factor 1 [DMRT1]), and induces proliferation of the germ cells that then redirect the already committed male somatic cells toward female development causing full sex reversal or intersex which is also seen in a variety of other fish species exposed to estrogen. A few laboratory studies have also examined the reversibility of some of the induced effects, such as those on VTG production in males and on intersex induction. These studies seem to suggest that feminization of the germ cells is, in some cases, reversible when exposed subjects are transferred to clean water, but that the overall fertility of these "reversed males" never reaches that of the true males and that courtship behaviour is incomplete.

Although few studies have examined the endocrine control of copulatory organ development and growth in nonmammalian species, what is known suggests that the development and growth of the phallus of reptiles and the gonopodium/genital papillae of fish is androgen-dependent (e.g. for reptiles see Raynaud & Pieau, 1985; fish, see van Tienhoven, 1983). Thus, if these species are exposed to EDCs with antiandrogenic activity, the androgen-dependent phallus would be developmentally altered and feminized. This could occur not only through the actions of environmental anti-androgens, but also through the actions of estrogens as well. In fish, for example, juvenile goby experimentally exposed to 17β-estradiol for 11 to 32 weeks exhibited signs of feminization of the genital papilla, showing that it was inducible by estrogenic exposure and could therefore be a form of estrogenic endocrine disruption. The estuaries where this condition was most prevalent (>50% at certain sites) were also those where estrogenic contamination was the most prominent.

The extensive literature on fish has revealed that the extent and severity of effects varies considerably between species, indicating a species-specific sensitivity to EDC

exposure. In this respect, a study of the incidence of intersex in the lower Great Lakes region, revealed that certain fish species sampled from the same site did not show any gonadal abnormalities (e.g. goldfish, carp, gizzard shad, brown bullhead, pumpkinseed and bluegill), whilst up to 45% of white perch were affected (Kavanagh et al., 2004). This pattern was also borne out during a whole lake study in Canada. Kidd et al. (2007) dosed an experimental lake with the steroid estrogen, ethinylestradiol (EE2; at a concentration of 5 ng/L), to assess the effects on VTG and gonad histology and, subsequently, population sustainability. The data revealed that, whilst VTG concentrations in male fathead minnow, pearl dace and lake trout were dramatically increased (by 1 900-24 000-fold), the effects were much less marked in male white sucker (by up to 118-fold; Palace et al., 2009). Although this scenario is not completely representative of a natural system in which fish are continuously exposed to a mixture of EDCs at low effect levels, the data provide very strong evidence that chemical exposure is associated with a suite of male reproductive abnormalities (intersex and abnormal spermatogenesis), compromising their reproductive capabilities and ultimately leading to the collapse of a "wild" population (Kidd et al., 2007).

2.3.3.3 Invertebrates

Compared with vertebrates, little is known about the manifestation of endocrine disrupting effects on the reproductive system of male invertebrates. However, there are some historical reports in which populations have exhibited signs of feminization, apparently in association with exposure to EDCs. For example, copepods, a type of minute crustacean, living near a long-sea sewage outfall in Inverkeithing, Scotland, were found to have higher than expected rates of intersex, a phenomenon which persisted up to 10 miles (16 km) from the outfall. No evidence was found of disease or parasitism that could have accounted for this phenomenon (Moore & Stevenson, 1991). Intersex has also been reported in lobsters living near sewage outfalls (Sangalang, 1997). More recently, intersex has been reported in molluscan species; following the oil spill from the *Prestige* oil tanker in 2002, populations of Mediterranean mussels in the estuary of the Oka River, in the Bay of Biscay, were found to have a high prevalence of intersex (26%). The area is also subject to pollution from industrial activity (metallurgic industry, ship building, foundries and cutlery making amongst other things). Intersex was absent in mussel populations from a nearby unpolluted area unaffected by the oil spill (Ortiz-Zarragoitia & Cajaraville, 2010). Intersex has also been reported in the estuarine bivalve, *Scrobicularia plana*, in UK estuaries; varying degrees of intersexuality were reported in over 20% of individuals sampled from 17 out of 23 populations and this was putatively linked to exposure to EDCs (Chesman & Langston, 2006). However, as a whole, field-based evidence of endocrine-mediated reproductive disorders in invertebrate males is scarce and solely concerns aquatic Crustacea and molluscs. Much more data are needed on other phyla and on terrestrial species. As a result, it is not yet possible to draw parallels with vertebrates with regards to the likelihood of a testis dysgenesis syndrome, although this remains a possibility.

2.3.4 Evidence for a common cause of male reproductive endocrine disruption in wildlife and humans

Taking all of the evidence together, it is clear that the patterns disturbance of male reproductive health in humans have clear analogies in wildlife: male alligators living in Lake Apopka, which exhibited a similar suite of male reproductive abnormalities following exposure to pesticides from a chemical spill (see section 2.4.1.2) to those seen in similarly exposed laboratory rodents, provide a notable example. Moreover, there is evidence that with accidental and occupational exposures in both humans and wildlife, reproductive dysgenesis occurs in response to high environmental exposure levels. However, there is still a paucity of evidence as to whether the lower levels widely encountered in human populations pose a risk to male reproductive development, although in wildlife species there are examples of widespread dysgenetic male reproductive development linked to EDC exposure, for example in male fish throughout UK rivers (Tyler, Jobling & Sumpter, 1998).

In addition to the rodent studies already described, there is also laboratory-based evidence to support the assertion that EDCs are involved in the causation of male reproductive disorders in wildlife species; vast numbers of exposure experiments, involving the analysis of a wide range of species, have been reported in the literature over the past twenty years. In reviewing this evidence, it becomes apparent that it is difficult to make generalisations about the effects of a particular chemical, or group of chemicals, in terms of their mode(s) of action and/or potency. This is largely due to differences in the ways different species respond to EDCs, but is also confounded by experimental variability in factors such as diet, exposure regime and duration of exposure, as well as differences in the effect level and endpoint under investigation and the technical approaches employed. For example, differences in potency of up to 3-4 orders of magnitude have been reported in fish, depending on the species under investigation and the experimental methodology (see Brian et al., 2005). As a result of their influence on experimental outcomes, these factors can confound the risk assessment of the chemical(s) in question and ultimately make it difficult to decide upon the hazards that these chemicals pose. However, there is a strong consensus that many EDCs have the capacity to derail male reproductive development in the various wildlife species in which endocrine disruption has been studied, leading to the feminization and/or demasculinization of the male form.

Some of the most convincing evidence stems from the analysis of EDC impacts on fish, through which it has been possible to explore and replicate sexual disruption in wild populations under controlled conditions in the laboratory. Since the first discovery that caged male fish placed downstream of sewage treatment works in British rivers exhibited elevated

VTG levels and intersex (Purdom et al., 1994), there have been intensive efforts to identify the chemical(s) responsible. This research has focused on the steroid estrogens and there is now unequivocal evidence from a wealth of laboratory-based studies demonstrating their capacity to feminize fish, with effects reported at all levels of biological organisation, from the molecular level through to impacts on reproductive capacity and population dynamics. However, many other types of chemicals that are also present in STW effluents have also been found to mimic the actions of the steroid estrogens, thus contributing to sexual disruption in fish. Although most of these chemicals, such as the alkylphenols (e.g. nonylphenol), are only weakly estrogenic and are generally found in the environment at low and individually ineffective concentrations, there is now convincing evidence that they can act together in combination with other chemicals that have the same mechanism of action (Thorpe et al., 2003; Brian et al., 2005; 2007). Moreover, it has recently been suggested that sexual disruption in wild male fish may not occur exclusively in response to estrogens and is, instead, a function of combined exposure to chemicals that act via the androgen receptor as well as those with estrogenic properties. A statistical modelling approach has been used to demonstrate that feminizing effects in wild fish can be best modelled by taking account of their predicted exposure to both antiandrogens and estrogens in STW effluents, as opposed to estrogens alone (Jobling et al., 2009). Although this theory is plausible, there are currently a paucity of laboratory-based data on the influence of anti-androgens on the reproductive development of fish and in view of the prevalence of anti-androgenic chemicals in the environment and their reported effects on laboratory rodents, further data addressing this issue in fish are needed.

Thus, it is clear that laboratory-based studies have contributed greatly to the evidence that EDCs are involved in the causation of male reproductive disorders in humans and wildlife. Although differences in sensitivity have been reported, in general, it would appear that the same chemicals, or groups of chemicals, elicit similar response patterns, regardless of the species in question and the test system used. Laboratory-based studies using rodents and non-mammalian species, and most notably fish, have been invaluable in demonstrating the capacity for EDCs to affect reproductive development at low and environmentally-relevant concentrations and in helping to identify critical periods of exposure during development. The data generated support the theory concerning the involvement of EDCs in the causation of male reproductive disorders in wildlife and, in many cases, mirror the evidence concerning the etiology of TDS in the humans. However, until recently, there has been an anomaly in the evidence in support of the hypothesis that effects in humans and wildlife have a common causation; the symptoms of TDS are most easily reproduced in rodent models by exposure to mixtures of antiandrogenic chemicals, whereas the feminization of wild male fish has been attributed mainly to exposure to steroidal estrogens. This casts some doubt on the concept of a shared etiology. However, recent studies indicating widespread anti-androgen presence in rivers and estuaries add credence to the hypothesis that the effects seen in both wild fish and humans may be caused by similar combinations of endocrine-disrupting chemical cocktails to which they are exposed (Jobling et al., 2009). If supporting laboratory-based data can be produced, this gap between the human and fish literature will ultimately be bridged.

It is important to recognise that some components of testis dysgenesis syndrome are not comparable across all species. Using cryptorchidism as an example, it is clear that, whilst widely reported in humans and many other mammals, this condition is not universally applicable to all mammalian species (e.g. elephants and marine mammals do not develop a scrotum and the testes are either held in an abdominal or inguinal location), or indeed to any species of reptile, fish, bird or amphibian. Within these classes, the testes are maintained within the body wall and, thus, do not exhibit testicular descent. However, situations such as this, in which there are clear differences in the structure of the reproductive system across classes and taxonomic groups, it is possible that the symptoms of endocrine disruption may still occur, but in a form that does not, at first, appear to be comparable with previously reported effects. For example, whilst the feminization of the males external genitalia may be characterized by hypospadias in mammals, in some species of fish, it may be that the same phenomenon is manifested by the abnormal development of the urogenital papillae (UGP), a structure that is normally well developed in males (like a penis), but less so in females. Evidence of feminized UPG (known as morphologically intermediate papilla syndrome; MIPS) has been reported in wild-caught sand gobies from contaminated sites around the coast of the UK (Kirby et al., 2003), as well as in sharp tooth catfish inhabiting an estrogen polluted freshwater source in South Africa (Barnhoorn et al., 2004). This provides an interesting parallel with hypospadias, despite the fundamental differences between the genital structure and morphology of mammals and fish. Alternatively, it is important to note that, due to the greater plasticity observed in wildlife, additional endpoints may be affected that have no clear analogy in humans. However, these symptoms could still form part of the same underlying syndrome and, thus, have the capacity to inform the overall understanding of testicular dysgenesis in other forms of wildlife, as well as in the human population.

From the above, it is apparent that most of the symptoms associated with TDS in humans, namely genital malformations and poor semen quality (along with depressed sex hormone concentrations), have also been reported in wildlife, although the wildlife literature clearly encompasses a much larger and more diverse range of symptoms. However, there is a major exception: testis germ cell cancer. Whilst this disease forms an important component of TDS in men, this symptom has not been associated with contaminant-induced endocrine disruption in any wildlife study to date. This may be due, at least in part, to the logistical difficulties in detecting this disorder in wild animals (Edwards, Moore & Guillette, 2006), combined with the likelihood of a low rate of occurrence (its incidence in the

human population is only around 1%). That is not to say that TGC cannot occur in any other species besides humans. Indeed, there are some highly suggestive cases in which atypical germ cells resembling the CIS cells found in human testes have been reported in domestic and laboratory animals, including horses (Veeramachaneni & Sawyer, 1998) and rabbits (Veeramachaneni & Vandewoulde, 1999). CIS is a pre-invasive precursor of TGC, the most common cancer type of human male adolescents and young adults (Rajpert-De Meyts, 2006). There is also growing evidence of abnormal testicular development in wild mammals. In this respect, a variety of testicular tumours, along with microlithiasis and CIS, have been detected in Sitka black-tailed deer on Kodiak Island, Alaska, which were suspected to have been developmentally exposed to estrogenic chemicals (Veeramachaneni, Amann & Jacobson, 2006). In addition, atypical germ cells were encountered in the testes of wild eland in South Africa, although detailed morphological examination for CIS was not possible (Bornman et al., 2010). Testicular microlithiasis and neoplastic lesions were also reported in these animals, which was coincident with high body burdens of environmental pollutants, in particular, alkylphenols.

Data from laboratory-based studies also support the chemical causation of these testicular abnormalities. For example, there is evidence that the developmental exposure of rabbits to a range of chemicals, including those with endocrine disrupting properties, produces symptoms of testicular dysgenesis in the form of atypical germ cells with features characteristic of CIS (Veeramachaneni, 2008). These findings indicate that a parallel for TGC does exist in other species of mammal and, furthermore, add credence to the hypothesis that EDCs are a factor in the increasing rate of TGC in the human population. In contrast, however, it may be that, for some classes of non-mammalian wildlife, TGC cannot occur due to absence of a similar mechanistic pathway.

2.3.5 Main messages

- Recent prospective studies indicate that chances of pregnancy decrease when sperm concentrations decrease below 40-50 million per mL and/or percentage of morphologically normal sperm declines below 9%.
- In a few countries (Denmark, Finland, Germany, Norway, and Sweden) where studies on semen quality in the general population have been systematically done, approximately 20-40% of men have suboptimal sperm concentrations and half of the men have less than 9% morphologically normal sperm. This most likely reflects recent declines in semen quality.
- These decreases in semen quality parallel increases in both the incidence of genital abnormalities in babies and the incidence of testis germ cell cancer in men in the same areas over the last 60 years. The occurrence of cryptorchidism at birth is associated with five-fold increased risk of testicular cancer, impaired semen quality and sub-fecundity.
- Increases in incidences of TGC, cryptorchidism and hypospadias and wide spread poor semen quality are most likely due to environmental factors. Exposures which interfere with the developing testis, including androgen action and/or production during fetal life, are likely to be crucial in the pathogenesis of TDS disorders. Other causes for poor semen quality are also known, such as genetic defects in sex chromosomes.
- Some epidemiological studies show weak associations between exposures to EDCs and the risk of cryptorchidism, hypospadias and decreased sperm production (occupational studies on greenhouse workers; chlorinated pesticides, PBDEs and dioxins).
- Exposures to several anti-androgenic endocrine disruptors have been shown to induce cryptorchidism, hypospadias and reduced semen quality in rodent experiments, often also linked to shortened anogenital distance.
- Wildlife are important sentinels for human male reproductive health as they are more easily sampled and live in direct contact with similar/the same complex mixtures of anthropogenic environmental contaminants to which humans are exposed. However, with the exception of fish, there are limited studies on reproductive abnormalities in males of other wildlife species.
- Symptoms of androgen deficiency and/or estrogen exposure also occur in a variety of wildlife species in both urban and rural environments and have been linked to exposure to contaminants in some cases.
- The symptoms of androgen insufficiency/androgen: estrogen imbalance are sometimes more severe than those seen in humans (i.e. developing eggs within the male testis of fish) because some non-mammalian species exhibit greater innate reproductive plasticity, and are thus more easily feminized.

2.3.6 Scientific Progress Since 2002

Since IPCS (2002), major advancements in our knowledge of endocrine disruption in males have occurred. These include:

- Testicular germ cell cancer has further increased increased in almost all countries in which it has been studied.
- Semen quality among 20-40% of young men from general populations in several European countries is in the sub-fertile range.
- An animal model for Testicular Dysgenesis Syndrome has been established in the rat, showing an inter-relationship between testicular dysgenesis and exposure to some EDCs during the fetal male programming window. There is now a mechanism via which irreversible disorders of the male reproductive tract can be caused.
- Various animal studies have confirmed that the fetus and the pre-pubertal animal are particularly sensitive to EDCs.

- Effects of estrogens in effluents from sewage treatment works on male fish have been seen in many countries and in several species of fish, indicating that this is a widespread phenomenon.
- The feminizing effects of estrogenic chemicals from sewage effluents on male fish, first reported in the 1990s, have now been seen in many countries and in several species of fish, indicating that this is a widespread phenomenon. Feminized (intersex) male fish have reduced sperm production and reduced reproductive success.

2.3.7 Strength of evidence.

There is sufficient evidence that male reproductive disorders, originating during fetal life, are increasing in the human populations in which they have been studied, and that this is partially related to environmental exposures. These diseases include cryptorchidism (testicular non-descent), hypospadias and testis germ cell cancer. There is also limited evidence linking these diseases and disorders with specific occupations and with exposures to chemicals with endocrine disrupting properties, particularly agricultural workers (pesticides and fungicides), PBDE flame retardants and phthalate plasticizers.

Prospective studies show that the occurrence of cryptorchidism at birth is associated with increased risk of testicular cancer, and impaired semen quality and sub-fecundity later in life, and there is limited evidence to suggest that this suite of male reproductive disorders (poor semen quality, TGC, cryptorchidism and hypospadias) are related components of a single underlying condition with a common etiology, termed testicular dysgenesis syndrome, originating during fetal development. There is, however, very little direct evidence for a role of endocrine disrupting chemicals in causing low semen quality in men following developmental exposures. Datasets that include both fetal exposures and adult measures of semen quality are rare.

There is evidence of suboptimal or poor semen quality in large proportions (20-40%) of men in countries in which this has been studied. There is even some evidence for a declining semen quality in these countries.

There is sufficient experimental evidence (from rodent models) supporting the hypothesis that androgen insufficiency during fetal/embryonic development could cause these male reproductive disorders and that EDCs that occur in our environment can contribute to the causation of these disorders.

With the exception of testis cancer (where the evidence is lacking), there is sufficient evidence in rodent models to support the hypothesis that phthalate plasticizers are causal factors in the manifestation of TDS but inadequate evidence to implicate these chemicals as the cause of TDS (or its separate entities) in humans or in any non-mammalian vertebrate.

The strength of evidence that EDCs occur in tissues at concentrations likely to cause endocrine disrupting effects as seen in the laboratory ranges from insufficient to sufficient (dependent on the case studied).

In some wildlife populations, there is sufficient evidence for developmental reproductive disorders and low semen quality in a proportion of the male animals, particularly in areas contaminated by anthropogenic contaminants with endocrine disrupting properties (primarily organochlorines, PBDEs and steroid estrogens). In non-mammalian vertebrate populations studied, the evidence for endocrine disruption is sufficient.

In wildlife also, a suite of effects often occur in concert and can be reproduced in laboratory studies by exposures to EDCs during early development. Taking the wildlife and human evidence together, there is a possibility that exposure to EDCs during fetal life and/ or during puberty plays a role in the causation of male reproductive health problems in humans, in some populations.

2.3.8 References

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2.4 Endocrine disrupting chemicals and sex ratio in humans and wildlife

2.4.1 Overview of endocrine disrupting effects on sex ratios in humans and wildlife

In humans, the sex ratio (numbers of boys divided by the numbers of girls born) is slightly greater than one, typically resulting in 51 to 52% boys being born (Allan et al., 1997; Astolfi & Zonta, 1999; Davis et al., 2007; Gutierrez-Adan, Pintado & De la Fuente, 2000; Moller, 1996; van der Pal-de Bruin, Veloove Vanhorick & Roeleveld, 1997). In vertebrate wildlife, however, the "normal" sex ratio may vary from one according to the life history of the animal; for example some fish species are first male and then female (or vice versa) and still other species are simultaneous or sequential hermaphrodites. Imbalances in sex ratios induced by exposure to chemicals may be the result of several processes: 1) the chemicals interfere directly with sex determination due to their endocrine properties inducing an increase in the number of individuals of a specific phenotypic sex, 2) the sperm cells or fetal stages of one of the sexes are more susceptible to the effects of a particular chemical than the other, resulting in lower conception or early death and thereby reduction in the number of individuals with the most vulnerable sex and subsequent gender imbalances, or 3) gender imbalances in laboratory experiments may be seen if exposure to a specific chemical promotes sexual maturation of one of the sexes. Given that estrogens and androgens participate in the phenotypic manifestation of genotypic sex, it is reasonable to question whether exposures to endocrine disrupting chemicals are influencing sex ratio in humans and wildlife. Available data suggest the following:

- Several researchers have reported recent small declines in the proportion of human male births in the USA, Canada, Denmark and the Netherlands.
- In addition, there are several specific cases of sharp alterations in the sex ratio of newborns associated with parental exposure to chemicals in industrial accidents or through their occupation.
- Feminized sex ratios induced by exposure to endocrine disrupting chemicals have been observed in a number of wild fish species exposed to the discharge of estrogenic wastewater effluents, whilst masculinized sex ratios are also observed below paper mill effluents and maybe in tributyltin-contaminated marinas. Laboratory studies corroborate these findings.
- There is also some evidence of changes in the sex ratio of some wild bird species, but this appears to be related to poor parental body condition. Similarly, some invertebrates are known to switch between parthenogenetic and sexual modes of reproduction when environmental quality declines.

2.4.2 Evidence for endocrine disruption of sex ratios in humans and in mammalian models of humans (rodents and primates)

Altered Sex Ratio Trends in Humans

During recent decades reduced proportions of male births have been reported in the populations or subpopulations of a number of countries, i.e. USA and Japan (Davis et al., 2007), Canada (Allan et al., 1997), The Netherlands (van der Pal-de Bruin, Veloove Vanhorick & Roeleveld, 1997), Spain (Gutierrez-Adan, Pintado & De la Fuente, 2000), Denmark (Moller, 1996) and in metropolitan areas in Italy (Astolfi & Zonta, 1999).

Mechanisms underlying sex determination in humans

The determination of sex is under stricter genetic control in mammals than in many other taxonomic groups. In an average population, slightly more boys (51-52%) than girls are born. The exact mechanism underlying this phenomenon is unknown.

The sex ratio in a population may vary in response to changes in several socio-economic factors, such as the nutritional status and the age of the mothers giving birth and the number of adult males in the population: thinner/malnourished and older mothers are less likely to have sons, whereas a low proportion of adult men in the population increases the probability of having sons (reviewed by Rosenfeld & Roberts, 2004).

Epidemiological evidence of a role for chemicals in causing alterations in sex ratio

Although the sex ratio is reported to be correlated with the age of mothers in some countries (e.g. Gutierrez-Adan, Pintado & De la Fuente, 2000), exposure to chemicals has also been suggested as a potential causative factor in declines in male births (van Larebeke et al., 2008). High occupational or accidental exposures to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Seveso (Mocarelli et al., 1996; 2000), to contaminated trichlorophenate in the saw-mill industry (Dimich-Ward et al., 1996), to the pesticide dibromochloropropane (reviewed by Goldsmith, 1997), or to PCBs (Hertz-Picciotto et al., 2008; Weisskopf, Anderson & Hanrahan, 2003) have all been associated with changes in the sex ratio of human populations. Moreover, lower sex ratios have also been reported for populations living in areas affected by air pollution from incinerators (Williams, Lawson & Lloyd, 1992) or industries (Williams, Ogston & Lloyd, 1995) and in a population potentially affected by the petroleum industry (Mackenzie, Lockridge & Keith, 2005). Overall, the results do not allow a robust conclusion regarding EDC influences on sex ratio in human populations, although the associations between chemical exposure and sex ratio are suggestive of this possibility.

Evidence for EDC effects on sex ratio in animal models

Studies on laboratory animals can shed light on sex ratio changes in the human population, such as the reduction in the percentage of boys (to 40%) born to mothers exposed to TCDD after the Seveso accident (Mocarelli et al., 2000). These studies reveal

that sex ratios in experimental mammals can be affected by a multitude of factors such as food availability, composition of the diet, and exposure to chemicals (reviewed by Rosenfeld & Roberts, 2004). In the Seveso case, whilst a three generation study of TCDD in rats found no effect on sex ratio in any generation of the treated animals (Rowlands et al., 2006), an experiment aimed at obtaining an exposure equivalent to the exposure following the Seveso accident found a decrease in the proportion of male offspring sired by exposed male mice (Ishihara et al., 2007). In a subsequent investigation, Ishihara et al. (2010) found no decrease in the Y-bearing/X-bearing sperm ratio, but the sex ratio of the 2-cell embryos of the TCDD exposed group was lower than that of the control group, indicating that the sex ratio of the offspring was decreased at fertilization, rather than at the spermatozoa stage.

2.4.3 Evidence for endocrine disruption of sex ratios in wildlife

2.4.3.1 Mammals

There are no documented examples showing changes in sex ratios related to exposure to EDCs in non-human mammals.

2.4.3.2 Non-mammalian vertebrates

Sexual determination and differentiation is generally more labile in non-mammalian vertebrates than in mammals. This is especially true in fish, amphibians and reptiles where a multitude of ambient factors (i.e. temperature, pH, population density, food availability, growth rate) may affect sex determination and differentiation. The exact genetic background for sex determination is not known for all species.

Skewed sex ratios induced by exposure to endocrine disrupting chemicals have been observed in a number of fish species in the field. The sex ratio in roach does not deviate from 1:1 in an uncontaminated environment (Allner et al., 2010; Geraudie et al., 2010), but controlled exposure to estrogenic wastewater effluents in tanks from one month post hatch up to 3.5 years of age resulted in 98% phenotypic females (Lange et al., 2011). Similarly, the discharge of estrogenic wastewater effluents caused a reduction in the percentage of male white suckers from upstream values between 36 and 46% to downstream values of 17 to 21% (Vajda et al., 2008). Nagler et al. (2001) found male genetic markers in phenotypically female Chinook salmon from the Columbia River, but in this case no direct link was made to endocrine disrupting chemicals.

In contrast, Larsson, Hollman & Förlin (2000) and Larsson & Förlin (2002) found sex ratios among the embryos of the viviparous blenny deviating from the normal 1:1 ratio in the vicinity of discharges from a pulp and paper mill in Sweden, with more male than female fish. A recent study of roach and perch, involving more than 3000 fish, which were caught at eight sampling sites, twice a year, in summer and late autumn/winter over a 2-year period found evidence of male-biased sex ratios. The sites, situated within the greater Upper Rhine catchment, were characterized by different degrees of anthropogenic impact. In addition, a significantly elevated proportion of male perch were obtained from a tributyltin-contaminated marina (Allner et al., 2010), although elevated proportions of male perch were also found at 3 sites with no documented contamination from tributyltin.

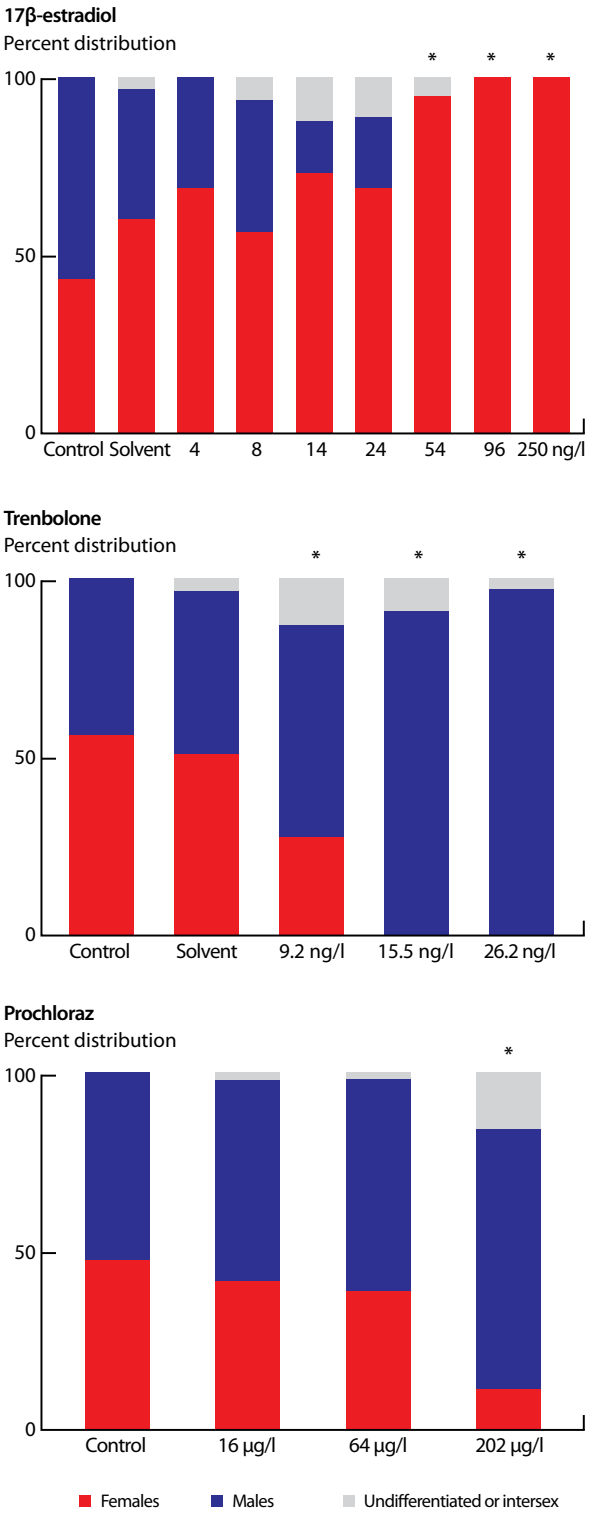
There is also some evidence of changes in the sex ratio of some bird species that are sexually size dimorphic, with males that are larger than females. Parents in poor body condition are able to switch their reproductive output towards the smaller sex. For example, a Norwegian colony of lesser black-backed gulls with high concentrations of organochlorines (PCBs, DDE, HCB, nonachlor, oxychlordane, heptachloroepoxide) in their blood had offspring with a sex ratio that was skewed towards females (Erikstad et al., 2009); no correlation was found between the blood concentrations of perfluorinated compounds or polybrominated diphenyl ethers and the sex ratio in the offspring, thus providing evidence for effects of specific chemicals and not others. The same phenomenon was expected in Arctic glaucous gulls (Erikstad et al., 2011) but, contrary to the expectation, females with high levels of organochlorines had offspring with a sex ratio strongly skewed towards males (most apparent among females in poor body condition). The mechanism underlying these changes in sex ratios is not known, but hatching failure in declining Swedish populations of tree sparrows was shown to result from embryo mortality, which was more likely to affect male embryos than females. The fledgling sex ratio was consequently highly female biased, however, the cause of this sex biased embryo mortality remains unknown (Svensson et al., 2007).

2.4.3.3 Invertebrates

Skewed sex ratios have been linked to exposure to EDCs in various mollusc species. Bacchetta & Mantecci (2009) found a higher proportion of female zebra mussels after a DDT-pollution incident in Lake Iseo, Italy, and Gagné et al. (2011) also found an elevated proportion of female freshwater mussels (*Elliptio complanata*) downstream of two municipal effluent outfalls in the Mille-Îles River (Quebec, Canada). At a tributyltin-contaminated site in the intertidal zone of the Saint Lawrence River (Quebec, Canada), Gagné et al. (2003) reported a decreased proportion of female softshell clams (*Mya arenaria*). Similarly, Leung et al. (2006) found an inverse correlation between the proportion of female neogastropods, *Thais clavigera*, and the body burden of TBT in Hong Kong waters.

2.4.3.4 Laboratory evidence for EDCs causing sex ratio changes in wildlife

Studies of sexual determination and differentiation in fish have revealed extraordinary sensitivity to external hormones or chemicals with endocrine properties (**Figure 2.8**). In the laboratory, populations of commonly-used test fish species such as zebrafish, Japanese medaka and fathead minnow can be made all female by exposure to estrogens during the period of sexual differentiation (Holbech et al., 2006; Nash et al., 2004; Zerulla



Sex ratios in zebrafish exposed to chemicals from hatch till 60 days after hatch when sexual differentiation is normally completed. Exposure to trenbolone (a potent synthetic androgen) and prochloraz (an aromatise inhibiting fungicide) masculinises the fish and the natural estrogen 17β-feminises them. Astrisks indicate significantly skewed sex ratios. Modified from Holbech et al. (2006), Kinnberg et al. (2007) and Morthorst et al. (2010).

Figure 2.8. Sex ratios of zebrafish exposed to chemicals (See legend in figure) (Figure reproduced with publisher's permission).

et al., 2002). In contrast, all male populations can be produced by exposure to androgens (Morthorst, Holbech & Bjerregaard, 2010) or aromatase inhibitors (Kinnberg et al., 2007; Zerulla et al., 2002). The sex ratio in these species is the dominant end point in OECD Test Guideline 234 (OECD, 2011), developed to identify endocrine disrupting chemicals. Sex reversal has also been induced experimentally in a number of other fish species, i.e. carp (Gimeno et al., 1996), Southern catfish (Liu, Zhang & Wang, 2010) and various salmonids (Piferrer, 2001).

Whilst there is extensive field-based evidence that exposure to estrogenic (and also possibly anti-androgenic) chemicals in sewage treatment works effluents is associated with female biased sex ratios (e.g. OECD, 2008; 2011; Lange et al., 2011; Vajda et al., 2008), a number of laboratory-based studies have demonstrated masculinizing effects of pulp and paper mill effluent also with effects on fish reproduction (e.g. Kovacs et al., 2011; Mower et al., 2011; Örn et al., 2006). Male bias was found in fathead minnows exposed to pulp and paper mill effluent during the period of

sexual determination and differentiation (Kovacs et al., 1995). The exact causative agent underlying this masculinizing effect of pulp and paper mill discharges is not known. Wastewater from pulp and paper mills contains complex mixtures of organic compounds including plant sterols with endocrine activity and, previously, when chlorine was used in bleaching processes, chlorinated dioxins and dibenzofurans were also discharged.

There is also experimental evidence that sex ratios can be affected in amphibians (Brande-Lavridsen, Christensen-Dalsgaard & Korsgaard, 2008; Pettersson & Berg, 2007) and reptiles (e.g. Bergeron, Crews & Mclachlan, 1994; Milnes et al., 2005) by exposure to hormones or chemicals with endocrine disrupting activity. Furthermore, laboratory-based experiments on birds have demonstrated that chickens are masculinized if the embryos are treated with an aromatase inhibitor (Yang et al., 2011).

Parthenogenesis, hermaphroditism, female-male cycling during life and gonochoristic sexual development illustrate the variability in sexual strategies among invertebrates, although the precise role of the vertebrate sex steroids within these organisms is not known. Most of the laboratory-based research into the effects of EDCs on sexual development has been done with arthropods, mainly crustaceans, where invertebrate-specific hormones, moulting hormone (ecdysone) and juvenile hormone (methyl farnesoate), also play important roles in development. Although the biochemical mechanisms are far from elucidated, laboratory studies suggest that exposure to EDCs may be able to change sex ratios among invertebrates.

The water flea, *Daphnia magna*, is a branchiopod crustacean, which is a common inhabitant in fresh water ponds in Europe and Asia. It is known to switch between parthenogenetic and sexual reproduction when environmental quality declines. The proportion of male *D. magna* increases upon exposure to atrazine (Dodson et al., 1999), the insecticides pyriproxyfen, fenoxycarb (Wang et al., 2005) and endosulfan (Palma et al., 2008), the miticide dicofol (Haeba et al., 2008), and the juvenile hormone methyl farnesoate (Rider et al., 2005; Wang et al., 2005). Whilst the insecticide methoprene (a juvenile hormone analog) has been reported to increase the number of males (Wang et al., 2005), it can also, conversely, reduce the number of all-male broods (Peterson, Uashian & Dodson, 2001). Similar contradictory results have been found for dieldrin in different species of *Daphnia* (Merritt et al., 1999; Wang et al., 2005).

In other invertebrate classes, bisphenol A exposure has been found to reduce the proportion of female houseflies (Izumi et al., 2008), and to increase the proportion of female isopods, *Porcellio scaber* (Lemos, Vab Gestel & Soares, 2009). The exposure of freshwater amphipods, *Gammarus pulex,* to EE2 resulted in a female dominated population (Watts, Pascoe & Carroll, 2002).

2.4.4 Evidence for a common cause of endocrine disruption of the sex ratio in humans and wildlife

The documented examples of EDC-related sex ratio imbalances in wild fish and invertebrates indicate direct interference of chemicals with sexual determination and/or differentiation, which is possible in these species. In contrast, it would appear that EDC-related sex ratio imbalances in bird populations are more likely to occur as a result of a negative selection of one sex. Humans may follow this model, with decreasing proportions of baby boys occurring as a result of negative selection on male embryos (or potentially sperm cells). Thus, the different mechanisms involved in sex ratio imbalances call for caution when making extrapolations from wildlife to humans about the causes of sex ratio imbalances.

2.4.5 Main messages

- EDC-related sex ratio imbalances resulting in fewer male offspring in humans do exist, e.g. in relation to dioxin and DBCP, although the underlying mechanisms are unknown.
- The effects of dioxins in humans are supported by results in experimental animals.
- EDC-related sex ratio imbalances have been seen in wild fish and molluscs and, in some of these species, are also supported by laboratory evidence.
- The mechanisms underlying EDC effects on sex ratios remain unknown for many species.

2.4.6 Scientific progress since 2002

The following progress has been made since the IPCS (2002):

- Skewed sex ratios in exposed fish and mollusc populations have been demonstrated.
- Skewed sex ratios in a dioxin-exposed human population have been corroborated by results obtained in a mouse model.
- In OECD Test Guidelines TG211 and TG234, sex ratios in *Daphnia magna* were included as endpoints to detect endocrine activity (OECD, 2008; 2011).

2.4.7 Strength of evidence

In wildlife, the changes in sex ratios are consistent with predictions from results of laboratory experiments for a number of the observed effects (e.g. more female fish downstream from estrogenic effluents than upstream) and the evidence that EDCs cause gender imbalances in some non-mammalian wildlife is sufficient. In the human population, the evidence that exposure to specific chemicals (e.g. dioxin and dibromochloropropane) causes gender imbalances in selected populations is sufficient. The evidence that the general decrease in sex ratios in a number of industrialized countries is related to exposures to EDCs is currently insufficient. However, based on the results of occupational exposures, it is plausible that exposure to EDCs can cause gender imbalances among humans.

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2.5 Endocrine disruptors and thyroid-related disorders and diseases

2.5.1 Overview of thyroid-related disorders and diseases in humans and wildlife and evidence for endocrine disruption

Thyroid diseases and disorders in humans (e.g. congenital hypothyroidism and adult autoimmune thyroid disease) have increased in incidence over the past several decades, such that the burden of thyroid disease is approximately two billion people worldwide.

- **Thyroid diseases and disorders represent a particularly high and increasing disease burden in children and adolescents** in several countries in which they have been studied (McGrogan et al., 2008).
- **Between 6-10% of adults have a thyroid disease or disorder.** Hypothyroidism is the most common thyroid disorder and is six times more common in women than men (Vanderpump, Turnbridge & French, 1995)
- **Population-wide testing of thyroid function in the absence of suggestive clinical features reveals a great proportion of "mild" thyroid abnormalities that have most likely gone unrecognized.** These studies suggest that there may be many more adults with undiagnosed thyroid conditions than is currently appreciated (e.g. Canaris et al., 2000).
- Slight decreases in thyroid function - sometimes referred to as "subclinical" or mild hypothyroidism - may have adverse health consequences (elevated cholesterol levels, heart disease and diabetes), especially over the long term and during pregnancy.

Both genetic and environmental factors play a role in thyroid health. However, observations in laboratory animals and wildlife suggest that exposure to endocrine disruptors, particularly during fetal life, could also play a role. Alongside the human health trends, studies describing thyroid dysfunctions in wildlife also exist. Sometimes, these wildlife observations are associated with exposures to contaminants. Examples include:

- **Relationships between body burden of persistent organic pollutants (PCBs, PBDEs and organochlorine pesticides) and thyroid-related effects in marine mammals;** in seals (Brouwer., 1989; Hall, Kalantzi & Thomas, 2003; Hall & Thomas, 2007; Routti et al., 2008), sea lions (Debier et al., 2005), beluga whales inhabiting the St. Lawrence estuary (DeGuise et al., 1995), the harbour porpoise (Schnitzler et al., 2008), and polar bear (Braathen et al., 2004, Skaare et al., 2001).
- **Significant thyroid disruption in monitoring studies of birds in the Great Lakes, Barents Sea, Tokyo Bay, linked with EDC (PBDE and PCB) burdens** (Scanes & McNabb, 2003; Verreault et al., 2004; Saita et al., 2004).
- **Thyroid disruption in salmonid fish living in heavily polluted regions of the Great Lakes in the United States during the 1970s and 1980s and, more recently, in mummichogs in New Jersey and San Francisco Bay** (reviewed in Jobling & Tyler, 2003; Zhou et al., 2000; Brar et al., 2010). Effects in mummichogs were positively correlated with PCB concentrations measured in the livers of the fish.

Hormonal mechanisms underlying thyroid disorders and diseases

The thyroid gland is located at the base of the throat and straddles the trachea. When it becomes physically enlarged in some diseases, it is visible to the eye or can be palpated (goitre). The major product of the thyroid gland is "thyroxine" (tetraiodothyronine, T_4). However, T_4 is not considered to be the most active form of the hormone; rather, it is converted to tri-iodothyronine (T_3), which then acts on the thyroid hormone receptor (TR) in cells.

Thyroid function itself is controlled by "Thyroid-Stimulating Hormone" (TSH, or "thyrotropin"). TSH is a large protein hormone secreted from the pituitary gland that binds to specific membrane receptors on thyroid cells and activates a biochemical pathway that stimulates thyroid hormone production and secretion (Taurog, 2004). The amount of TSH stimulation required to maintain blood levels of thyroid hormone within a "normal" range is controlled by a negative feedback relationship between serum T_4 and serum TSH (Larsen, Silva & Kaplan, 1981). The negative feedback action of T_4 occurs both at the level of the hypothalamus (Vella & Hollenberg 2009; Hollenberg 2008; Greer et al., 1993; Koller et al., 1987; Aizawa & Greer 1981) and pituitary (Wan, Farboud & Privalsky, 2005; Hodin et al., 1989; Chin & Carr, 1987; Carr, Need & Chin, 1987). Thus, under normal conditions, there is a negative correlation between serum levels of T_4 (specifically "free" T_4) and serum TSH.

For this reason, blood levels of T_4 and TSH form the principle clinical measures of thyroid function and disease. So-called "reference" ranges are developed for human populations because there are slight differences in the set-point around which thyroid hormone is regulated in different races, ethnicities and in pregnancy. These reference ranges are generated from a large sample of the population that is without other measures of thyroid disease (symptoms or the presence of anti-thyroid antibodies) (Haddow et al., 2004; Surks, 1991). Reference ranges have been developed for different populations (e.g. Zarkovic et al., 2011), for the different periods of pregnancy (Haddow et al., 2004), even for twin versus singleton pregnancy (Haddow, Palomaki & McClain, 2006), and for preterm versus term birth (Clark et al., 2001; Adams et al., 1995).

Thyroid hormones are important for normal development of the human brain (Bernal, 2007; 2011; Oerbeck et al., 2007), lungs (van Tuyl et al., 2004; Bizzarro & Gross, 2004), heart

(Stoykov et al., 2006; Grover, Mellstom & Malm, 2005; Danzi, Dubon & Klein, 2005), and other organs. Moreover thyroid hormones induce metamorphosis in some fish (Yamano et al., 1994) and in frogs (Buchholz, Paul & Shi, 2005), and they are essential for development in birds (McNabb, 2006) and mammals (Zoeller & Rovet, 2004). There is remarkable evolutionary conservation among vertebrates and some invertebrates in the chemistry of thyroid hormones, as well as in their role in development and adult physiology (Heyland, Reitzel & Hodin, 2004; Heyland & Moroz, 2005). Likewise, the molecular signalling pathways (involving thyroid hormone receptors) through which these hormones exert their actions are highly conserved across the vertebrate taxa (Buchholz, Paul & Shi, 2005; Bertrand et al., 2004; Whitfield et al., 1999).

Endocrine disruptors as risk factors in thyroid disease and dysfunction

Given the importance of thyroid hormone in human and wildlife physiology, and the life-long effects of thyroid dysfunction during development, it is reasonable to carefully consider the possibility that environmental chemicals may interfere with the ability of thyroid hormone to perform its functions. There is a very large list of environmental chemicals – mostly human-made – that can cause a reduction in circulating levels of thyroid hormone in experimental animals (Howdeshell, 2002; Brucker-Davis, 1998). Not all of these produce goitre, although they all reduce serum concentrations of thyroid hormone. Moreover, more environmental chemicals are being identified that can interfere directly with the receptor for thyroid hormone (Zoeller, 2010) or with other processes controlling thyroid hormone action (Gilbert et al., 2011; see **Figure 2.9**).

Thyroid hormone dependent mechanisms of nervous system development in animals and humans

Severe thyroid hormone deficiency produces severe brain damage (Chen & Hetzel, 2010) and moderate or even transient insufficiency can cause specific developmental defects in rodents (Auso et al., 2004; Crofton, 2004; Crofton et al., 2000; Goldey et al., 1995; Goodman and Gilbert, 2007), and in humans (Haddow et al., 1999; Kooistra et al., 2006; Oerbeck et al., 2003; 2007; Pop et al., 1995; 2003; Pop & Vulsma, 2005). Small differences (~25%) in point estimates of maternal T_4 or TSH during the early fetal period are associated with adverse outcomes in humans (e.g. reduced IQ scores), even though hormone levels are not outside the population reference range (Haddow, Palomaki & Williams, 2002; Morreale de Escobar, Obregon & Escobar del Rey 2000). However, in a hallmark study by Bongers-Schokking et al. (2000), the Mental Development Index of children with congenital hypothyroidism was affected by the age of onset of treatment with thyroid hormone, rather than the specific serum free T_4 concentration after treatment. Thus, the degree of

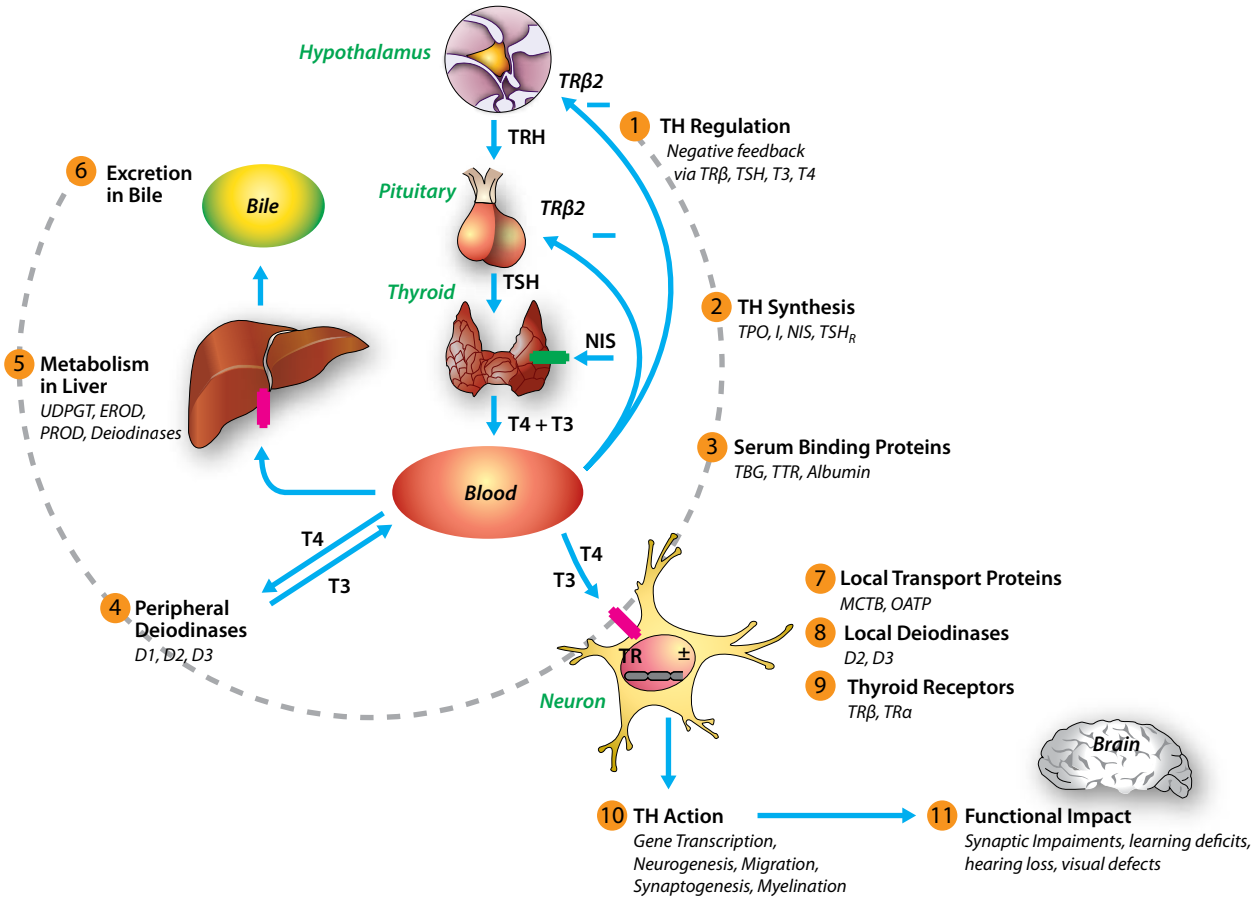

Figure 2.9. Possible sites of action of environmental contaminants on the HPT axis (Figure from Gilbert et al., 2011, redrawn; Used with publisher's permission).

thyroid hormone insufficiency is not the only variable affecting human development; the duration of the insufficiency and the developmental timing of the insufficiency are also important and may vary by species, presenting a challenge for risk assessment. This is discussed further in Chapter 1.2.4)

Experimental work in animals provides strong support for the hypothesis that moderate to mild thyroid hormone insufficiency can alter development in rodents. Integrating data over a series of studies, a decrease in serum total T_4 by 50% during the critical period for cochlear development in the ear was associated with a permanent hearing loss in adult offspring (Crofton, 2004). Moreover, Auso et al. (2004) found that less than a 30% decrease in serum total T_4 in female rodents, for only 3 days, was associated with structural abnormalities in the brains of their offspring. An average decrease in serum total T_4 of only 28% in 2-week-old pups given low doses of propylthiouracil was associated with marked reduction in cell density of the corpus callosum region of the brain (Sharlin et al., 2008). Interestingly, Gilbert & Sui (2008) found that a 28% reduction in circulating levels of T_4 in pregnant rats produced significant adverse effects on synaptic function of hippocampal neurons of their adult offspring despite no detected change in serum T_4 levels in the pups after birth. The US EPA has discovered a cluster of neurons that reproducibly migrates to an incorrect position in the brain of animals that have low thyroid hormone (Goodman & Gilbert 2007). Elements of this cluster very sensitive to prenatal thyroid hormone insufficiency have been characterized (a heterotopia) (Gilbert et al., 2012). Finally, Sharlin et al., found a very strong inverse relationship between serum T_4 in rat pups and the numbers of myelin-forming oligodendrocytes in major white matter tracks in the brain (Sharlin et al., 2008), and this was not compensated for by elevated serum TSH (Sharlin et al., 2010). Thus, experimental findings confirm what has been observed in humans: small, even transient, decreases in serum total T_4 are associated with altered brain development.

In general, there is strong evidence to conclude that thyroid hormone plays the same general role in brain development of animals and humans (Zoeller & Rovet, 2004). This clearly indicates that rodents represent important test systems to provide information important for protecting public and wildlife health from chemical exposures. In animal studies, the investigator is able to measure the effect of environmental chemicals on blood levels of hormones, and can fully characterize the consequences of these changes on thyroid hormone action at the molecular, cellular and tissue level at various times during development. In addition, a variety of drugs and genetic lines of mice are available to experimentally confirm that environmental chemicals are specifically disrupting thyroid hormone action and not some other pathway of toxicity that could produce similar effects on apical endpoints. In contrast, in human studies, the investigator can only correlate measures of hormone levels in the blood with exposures and with various metrics of health and very few additional measures can be obtained to help interpret the relationship between these variables of interest. Therefore, it is critically important to consider animal studies in the interpretation of human studies.

Notwithstanding this, the current set of validated test methods in the USA and EU for evaluating the ability of chemicals to interfere with thyroid hormone action does not include testing whether the chemical can interfere with thyroid hormone action (Zoeller, Tan & Tyl, 2007a; Zoeller, Tyl & Tan, 2007b).

2.5.2 Evidence for endocrine disruption of the thyroid in humans and in mammalian models of humans

2.5.2.1 Human thyroid diseases and disorders

Thyroid disorders are amongst the most prevalent of medical conditions and include goitres or thyroid nodules (adults), congenital and adult hypothyroidism, autoimmune thyroiditis, hyperthyroidism or Graves' disease and thyroid cancer. In this section, we will deal mostly with congenital and adult hypothyroidism as well as Graves' disease, the remainder being covered in sections 2.11 (autoimmune diseases) and 2.7 (thyroid cancer). As already mentioned, thyroid hormone deficiencies during the development of the brain can also cause neurodevelopmental disturbances leading to mental difficulties, manifest as Attention Deficit Hyperactivity Disorder (ADHD), learning difficulties and possibly even autism. These are discussed further under section 2.6.

Hypothyroidism: This refers to an "underactive" thyroid gland such that it produces too little thyroid hormone. Symptoms associated with hypothyroidism are broad and can be somewhat non-specific including cold intolerance, weight gain, lethargy, and low mentation (Haddow, 2010). Moreover, the body consumes less oxygen and produces less body heat. Hypothyroidism can occur in both children and adults. In the adult population, studies in Northern Europe, Japan and the USA have found the prevalence of hypothyroidism to range between 0.6 and 12 per 1000 women and between 1.3 and 4.0 per 1000 men investigated, although the prevalence is higher in surveys of the elderly (Vanderpump, 2011).

Congenital hypothyroidism: Congenital hypothyroidism (CH) is one of the most common preventable causes of mental retardation caused by thyroid dysgenesis during fetal life. In the first trimester, the fetus is dependent on the trans-placental passage of thyroid hormones of maternal origin because the fetal thyroid gland does not produce thyroid hormone until the end of the first trimester, and then in sufficient quantities only at 20 weeks gestation (Smallridge et al., 2005). Thereafter, however, a hypothyroid fetus will synthesize around 70% less T4 than a normal fetus leading to CH (Olney, Grosse & Vogt, 2010). In 75-80% of all cases of CH, the underlying etiology is unknown, whilst the remaining 15-20% have genetic thyroid dyshormonogenesis. A daily iodine intake <25 µg, particularly in preterm infants, accounts for many cases of CH in Europe, Asia and Africa, but multiple other factors may also be causal elements.

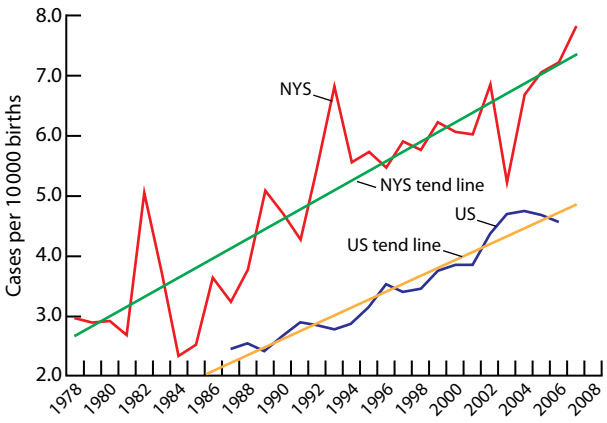

Figure 2.10. Incidence rate of CH in New York State (NYS), 1987–2007, and in the United States (excluding NYS), 1987–2006. (Figure from Hinton et al. (2010), redrawn; Used with publisher's permission).

Estimates of the birth prevalence of congenital hypothyroidism (CH) varies considerably throughout the world where universal screening programs are in place, as reviewed by Rendon-Macias et al. (2008). These estimates range from 1:1403 in Iran to 1:6450 in Latvia.

It was recently reported that the incidence of congenital hypothyroidism has nearly doubled over the past two decades in several countries in which it has been studied including the USA (Harris and Pass 2007; **Figure 2.10**), Western Australia (Kurinczuk et al., 2002), Italy (Corbetta. et al., 2009), the northern UK (Pearce et al., 2010b), and Greece (Mengreli et al., 2010). Some authors speculate that this is due to changes in the cut-off values for the neonatal screening system in the definition of this disorder (Mitchell, Hso & Sahai, 2011; LaFranchi, 2011). This will be an important issue to address.

Subclinical hypothyroidism (mild thyroid failure)

More widespread testing of thyroid function in the absence of suggestive clinical features suggests there are a great number of individuals, not diagnosed with thyroid problems, in which only TSH is abnormal (see **Figure 2.11**). A population study in Colorado, of over 25, 000 individuals of mean age 56 years, showed TSH excess in 9.5 % of the population and suppressed TSH in 2.2%; over half the group with suppressed TSH were taking thyroid medication. Similarly, in the Whickham survey in North East England, 8% of women and 3% of men had subclinical hypothyroidism and in the National Health and Nutrition Examination Survey (NHANES III), approximately 2% of adolescents aged 12–19 years had a serum TSH >4.5 mIU/L.

Prevalence data from one region do not necessarily apply to other populations, because of differences such as ethnic predisposition or variations in iodine intake. Several European studies have compared the effect of various levels of iodine intake on the prevalence of thyroid over- and under-function. Hypothyroidism is generally more common with abundant iodine intake, while goitre and subclinical hyperthyroidism are more common with low iodine intake.

2.5.2.2 Evidence for EDC exposures causing thyroid diseases and disorders.

It is possible that specific chemical exposures could lead to clinical thyroid disease and that this could be reflected in observed secular increases in the incidence or prevalence of thyroid disease. As reviewed above, thyroid disease is defined in large part by the presence of blood levels of T_4 and TSH that are outside the population reference range. For example, clinical hypothyroidism is defined as low T_4 and high TSH; both hormones need to be outside the reference range. However, the clinical symptoms associated with this hormone profile are highly variable in the population, and

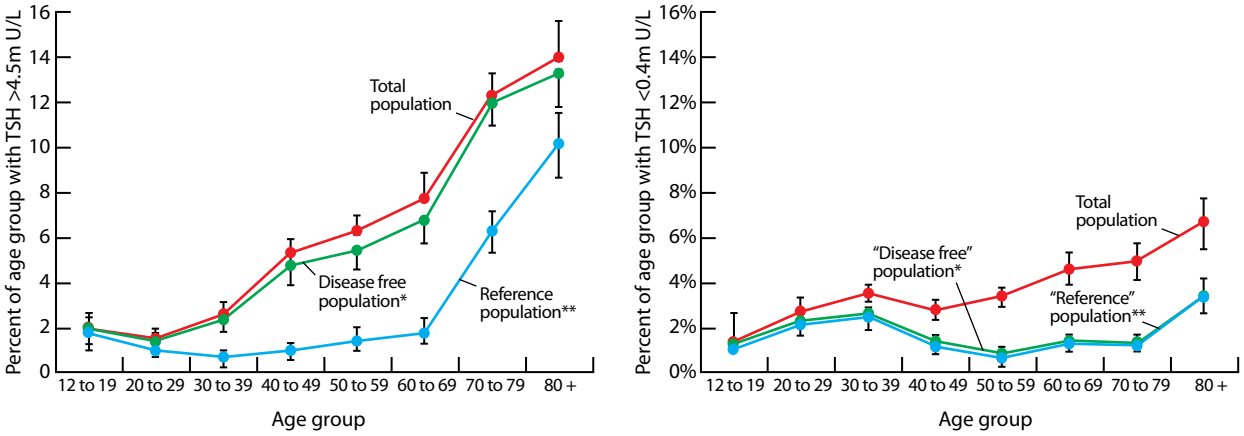

Figure 2.11. Percentage of the USA population (in 2002) with abnormal serum TSH concentrations as a function of age. The disease-free population excludes those who reported thyroid disease, goiter or thyroid-related medications; the reference population excluded, in addition, those who had positive thyroid autoantibodies, or were taking medications that can influence thyroid function. Note the much higher prevalence of TSH abnormalities in the total population, than in the reference population (Figure from Hollowell et al. (2002), redrawn; Used with publisher's permission).

as a result, a significant proportion of the general population can have undiagnosed thyroid disease. In fact, in addition to the 14 million adults in the USA with diagnosed thyroid disease, a further 13 million are estimated to be undiagnosed (Blackwell, 2004). With such a large proportion of likely undiagnosed disease, it is clear that reported changes in incidence or prevalence would not be meaningful. Moreover, because thyroid hormone levels are variable within individuals (Andersen et al., 2003; Andersen et al., 2002), it will be difficult to identify relationships between clinical disease and chemical exposures; in contrast, it may be more likely that chemical exposures will be related to thyroid hormone levels within the reference range. Risk assessors should not disregard such relationships for several reasons:

- First, a large number of chemicals can affect circulating levels of thyroid hormone in animals (Howdeshell, 2002; Brucker-Davis, 1998). Although there are differences between rodents and humans in some characteristics of the thyroid system (see below), rodent systems still provide important fundamental information for the pharmaceutical development of therapeutics for humans. Therefore, it seems inefficient to employ rodent systems to develop drugs but to fail to use rodent systems to protect public health.
- Second, serum TSH levels within the reference range have been identified as a risk factor for blood pressure and serum cholesterol (reviewed in Miller et al., 2009) as well as for bone in postmenopausal women (Morris, 2007). This suggests that serum thyroid hormone levels – TSH and possibly total or free T_4 – will be useful measures to link chemical exposures to various diseases.
- Finally, small differences in serum thyroid hormone levels during pregnancy or at birth are associated with deficits in cognitive function (LaFranchi, 2010). Therefore, if the fetus or neonate is as sensitive to chemical exposures as are adults, then even weak relationships between chemical exposure and hormone levels could produce permanent adverse effects.

A comprehensive review of this literature has recently appeared (Boas, Feldt-Rasmusssen & Main, 2011; Boas, Main & Feldt-Rasmusssen, 2009). There is now reasonably firm evidence that PCBs have thyroid-disrupting effects and that several other common contaminants also have such properties. These include brominated flame retardants, phthalates, bisphenol A and perfluorinated chemicals. In all cases, chemical exposure has been associated with serum thyroid hormone levels. Chemicals may affect circulating levels of thyroid hormone by interacting with the thyroid system in different ways (**Figure 2.9**) and there is currently little information about exactly how these may interact. A key issue is the extent to which changes in circulating levels of thyroid hormone reflect changes in thyroid hormone action in tissues (e.g. Zoeller, 2003). Human exposure to these chemicals (listed in Chapter 3, Table 3.1) is comprehensively reviewed in Chapter 3.2.2.

2.5.2.3 Polychlorinated biphenyls (PCBs)

PCBs are a family of biphenyls that have been randomly chlorinated, producing a mixture of chemicals that have as many as 209 different chlorination patterns. Their biological activity is altered by these patterns; in general, chlorination patterns that stabilize the ring structures into a planar conformation have dioxin-like activity (Kafafi et al., 1993; Kafafi et al., 1992) and those whose chlorination pattern stabilizes the ring structures into a non-coplanar conformation have a variety of activities (Lyng, 2007; Zoeller, 2001; Seegal & Shain, Snyder-Keller & Seagal, 1992; Shain, Bush & Seegal, 1991). Although PCB production was banned in the 1970s, PCBs remain common contaminants in the environment and in humans and wildlife both because of their chemical stability and because of the widespread use from heavy industrial applications to home products such as floor finishes and window caulking.

A number of studies have reported associations between PCB exposure and measures of thyroid function in humans that support the hypothesis that PCBs can reduce circulating levels of thyroid hormone (Abdelouahab et al., 2008; Hagmar et al., 2001a; 2001b; Persky et al., 2001; Schell et al., 2008; Turyk, Anderson & Persky, 2007). Some studies indicate that PCB body burdens suppress serum T_4, whilst others indicate serum T_3. In some cases, the findings are in men, in other cases in women. Overall, it is not a uniform picture. In studies of pregnant women, PCB body burden is positively associated with serum TSH (Chevrier et al., 2007; Takser et al., 2005). Studies of newborns also indicate that PCB body burden suppresses thyroid function (Chevrier et al., 2007; Herbstman et al., 2008). However, a number of studies report no associations between PCB body burden and measures of thyroid function (e.g. Dallaire et al., 2009; Dallaire et al., 2008; Longnecker et al., 2000).

There are a very large number of variables that must be considered to identify a relationship of interest between PCB exposures and measures of thyroid function. These include the fact that PCBs have a very long half-life in the human body and that there are many different PCB congeners that could influence thyroid function differently. There are also slightly different congener profiles in different populations. Measures of thyroid function are also variable across the population (serum total and free T_4 and T_3 and TSH) and this is exacerbated when time-of-day (with which thyroid hormone levels vary) is not standardized. Likewise, there are small gender and population differences. In one study of newborns, the birth mode (caesarean versus vaginal delivery) was important in identifying a relationship between serum PCBs and measures of thyroid function (Herbstman et al., 2008).

Evidence for PCB exposures causing thyroid diseases and disorders in rodent models

Considering these issues, it should be expected that not all studies will find exactly the same relationships. The issue is whether observed correlations between PCB body burden and various measures of thyroid function are consistent with an effect on population health that is mediated by effects on

thyroid hormone action. This is where experimental studies in animal models can be revealing. PCB exposures nearly uniformly cause a reduction in serum total and free T_4 (Gauger, Sharlin & Zoeller, 2007a). However, serum TSH is not often reported to be elevated in response to this decrease (Hood and Klaassen). In addition, different PCB congeners appear to be differentially potent at causing serum T_4 reductions (e.g. Giera et al., 2011), although it is not clear why this is observed. In a controlled study comparing the effects of reduced serum T_4 produced by either propylthiouracil (PTU), which blocks thyroid hormone synthesis, or various PCBs, which presumably induce liver microsomes and decreases the serum half-life of T_4, Giera et al. (2011) found very different effects of PCB exposure compared to PTU exposure. Despite the fact that both exposures brought serum total T_4 to the same concentration in blood, the two exposures had very different effects on the expression of known thyroid hormone response genes in the liver. Thus, the effect of PCB exposure on serum thyroid hormone levels cannot be interpreted the same way as the effect of PTU on serum thyroid hormone levels. This conclusion is supported by other studies (Bansal & Zoeller, 2008; Roegge et al., 2006; Bansal et al., 2005).

These findings also indicate that PCBs, or at least some congeners or metabolites, can interact directly with the thyroid hormone receptor. This hypothesis has been supported by a variety of studies. Several hydroxylated PCBs have been shown to displace T_3 from the TR (You et al., 2006; Kitamura et al., 2005), or to increase (Freitas et al., 2011; Gauger et al., 2007) or decrease (Amano et al., 2010; Miyazaki et al., 2008) thyroid hormone receptor activation in expression systems. Likewise in vivo, PCBs produce effects that are consistent with the hypothesis that they can interfere with thyroid hormone action; in a recent study, PCB body burden in killer whales was highly correlated with the expression of the thyroid hormone receptor (Buckman et al., 2011), a known target of thyroid hormone itself.

Taken together, these findings reveal relatively inconsistent relationships between PCB exposure and measures of thyroid function in humans, but very strong evidence in animals and in molecular studies indicating that PCBs can interfere with thyroid hormone action. The complexity of the human data has been interpreted by some to indicate that there is no convincing evidence that PCBs interfere with thyroid function in humans (Kimbrough & Krouskas, 2003). Moreover, these authors suggest that even if the current data indicate that PCBs can interfere with thyroid function in humans, it is not clinically relevant. Importantly, this review did not include aspects of thyroid measurements that would provide insight into the difficulty in observing PCB effects of interest or the kind of statistical analysis that would be required.

All studies of endocrine disruptors in humans will likely have elements of the dataset observed with PCBs. Specifically, environmental chemicals may produce effects on endocrine systems that are either dissimilar to that of overt disease states, or that are inconsistent from one study to the next due to the difficulty in standardizing exposure measures and measures of hormone levels.

2.5.2.4 Other environmental chemicals

Boas, Feldt-Rasmussen and Main (2011) have also reviewed the literature linking a variety of chemical exposures to thyroid function in humans. These include PBDEs, pesticides, perfluorinated chemicals, phthalates, bisphenol A, UV-filters and perchlorate. With the possible exception of perchlorate, none of these chemicals have been as extensively for their relationship to thyroid function as that of PCBs. Human exposure to these chemicals is, however, extensive (Chapter 3.2.2). Suvorov and Takser (2008) suggest that the PCB story can further inform the number of publications (and time) required to generate enough data to make informed decisions about human and wildlife health.

2.5.2.5 The perchlorate controversy

Perchlorate is an oxidant used in a variety of industrial applications, from the production of solid rocket fuels, to explosives used in automobile airbags, fireworks and blasting caps (reviewed in Oxley et al., 2009). Perchlorate is also naturally occurring (Dasgupta et al., 2006), though the relative degree to which environmental contamination is caused by human-made or naturally occurring perchlorate is not clear. Perchlorate is chemically stable when wet and persists for long periods in geological systems and in groundwater. Largely because of disposal practices during the 1960s – 1990s, perchlorate became a common contaminant of groundwater in the United States (Urbansky, 2002).

The best known biological effect of perchlorate is the inhibition of iodide uptake by the sodium/iodide symporter (NIS) (Wolff, 1998), although it has recently been reported that perchlorate also interacts with Pendrin, another iodide transporter (Attanasio et al., 2011). NIS is responsible for transporting iodide into the thyroid gland, which is required for the production of thyroid hormone (Carrasco, 2000). In addition, this protein is expressed in the gut (Nicola et al., 2009; Vayre et al., 1999), lactating breast (Nicola et al., 2009; Dohan et al., 2003; 2007), placenta (Mitchell et al., 2001), and choroid plexus (Carrasco, 2000), all presumably as a delivery mechanism for iodide to the thyroid gland. In this regard, it is important that the expression of NIS in the human fetal thyroid gland is the limiting step in the production of thyroid hormone (Szinnai et al., 2007).

Given the essential role of thyroid hormone in development, it is important to determine whether perchlorate exposure is associated with measures of reduced thyroid function in the human population. Early studies sought to test this by comparing T_4 or TSH levels in blood spots taken as part of the neonatal screening program with a proxy measure of perchlorate exposure – i.e. the city in which the infant was born (Las Vegas compared to Reno, Nevada, USA) (Li et al., 2000a; 2000b; Crump et al., 2000; Lamm and Doemland, 1999). The hypothesis was that because municipal drinking water was contaminated with perchlorate in Las Vegas but not in Reno, pregnant women and neonates would be exposed to perchlorate in Las Vegas but not in Reno. These studies uniformly found no association between the city of birth and neonatal thyroid

hormone. This was further supported by studies in Chile, in which perchlorate of natural origin is high, and again found no association between neonatal measures of thyroid function and the city of birth (Crump et al., 2000; Tellez Tellez, 2005). It was later shown in national biomonitoring data that almost everyone in the USA is exposed to perchlorate on a continual basis (Blount et al., 2006a; 2006b) and that much of this is derived from food (Huber et al., 2010; Sanchez et al., 2009). Therefore, studies using point estimates of exposure (i.e. city of birth) were confounded by large misclassifications of exposure and provide little useful information concerning the relationship of interest, i.e. perchlorate exposure and thyroid function.

A separate series of studies were performed to determine the efficacy of perchlorate exposure on iodide uptake inhibition in human volunteers (Greer et al., 2002; Lawrence, Lamm & Braverman, 2000; 2001), with the idea that this would help determine whether human exposures could influence thyroid function in the general population. These studies indicated that adults would have to consume 2L of drinking water daily that was contaminated with at least 200 ppb (µg/L) perchlorate to reach a level in which iodide uptake would begin to be inhibited (Greer et al., 2002). Of course, the relationship between iodide uptake inhibition, thyroid hormone synthesis and serum concentrations of thyroid hormone is not known, but was believed to require significant iodide uptake inhibition for extended periods before thyroid function would be impaired. Based on these studies, a USA National Academy of Science (NAS) committee recommended a reference dose (RfD) of 0.0007 mg/kg per day (National Research Council, 2005), which the US EPA used to set a provisional drinking water standard of 15 ppb.

Several authors disagreed with EPA's drinking water standard of 15 ppb and perchlorate remediation goal of 24.5 ppb on the basis that it did not consider infants (Ginsberg et al., 2007). The reason for this was that infants must synthesise their supply of thyroid hormone each day (van den Hove et al., 1999); thus, if environmental factors reduce thyroid hormone synthesis and hormone levels decline, adverse effects on cognitive function would develop. Infants are very sensitive to thyroid hormone insufficiency (Zoeller & Rovet, 2004) and small differences in circulating levels of thyroid hormone in infants have been associated with differences in measures of cognitive function into adulthood (LaFranchi & Austin, 2007; Oerbeck et al., 2003; Heyerdahl & Oerbeck, 2003). Ginsberg et al. (2007) calculated that as many as 90% of nursing infants may exceed the RfD, although later empirical measurements indicate that this number is probably closer to 10% (Valentin-Blasini et al., 2011).

Blount et al. (2006b) showed a significant and sizable association between urinary perchlorate and serum thyroid hormones in a statistically representative sample of the USA population as part of the NHANES survey. This association was observed for women, but not for men. Importantly, the associations observed are plausibly consistent with a cause-effect relationship. That is, urinary perchlorate was positively associated with serum TSH and this association was stronger when urinary iodide was low. In addition, urinary perchlorate was negatively associated with serum T_4 levels when urinary iodide was low. Thirty percent of women in this study had low urinary iodide (below 100 µg/L). Using this same dataset, Steinmaus, Miller & Howd, (2007) showed that women who smoked had elevated levels of thiocyanate, which also inhibits iodide uptake by the NIS, and that in women with low urinary iodide, the association between perchlorate exposure and measures of thyroid function was much stronger.

Conclusions from the NHANES 2001-2002 data are not easily reconciled with the earlier studies of human volunteers or with other population studies (Pearce et al., 2010a; 2011). If these studies reflect a true relationship between very low levels of perchlorate exposure and thyroid function, it would mean that data derived from short-term, high-dose experiments in humans do not accurately predict effects of chronic low-dose exposures.

The conflicting findings among epidemiological studies of the relationship between perchlorate exposure and thyroid function should highlight features of the thyroid system that do not appear to be commonly taken into consideration. One of the most important of these is that circulating levels of thyroid hormone are somewhat variable in each individual (Andersen et al., 2002). In fact, Andersen et al. (2002) estimate that it would require 25 separate tests to estimate the "set point" for serum T_4 in a single individual with a precision of 5%. Thus, the known variability in measurements of T_4 and TSH should be employed to estimate the number of subjects needed to test whether there is a relationship between serum T_4 and perchlorate. Likewise, consideration needs to be given to the known variability of estimates of perchlorate exposure. None of the current studies formally calculate the number of participants that would be required to identify a relationship between serum T_4 (or TSH) and urinary perchlorate. The Blount study included over 1,111 women in their study – the largest to date.

The story of perchlorate contamination should be used to inform studies of other contaminants and their relationship with thyroid function. For those exposures that will act by changing circulating levels of thyroid hormone, perchlorate can serve as a direct example and it will be important to ensure that the study has enough subjects to provide adequate statistical power. This is important because there are known associations between circulating levels of thyroid hormone in pregnant women and, especially, neonates that provide very strong evidence linking hormone levels to adverse outcome. However, for exposures to chemicals that can interfere with thyroid hormone signalling without affecting serum hormone levels, there is clearly a lack of approach at this moment to test these associations in the human population.

2.5.3 Thyroid hormone and other organ systems

It is important to recognize that thyroid hormone concentrations are correlated with adverse effects in organ systems other than the nervous system in the adult, including the cardiovascular system and control of serum lipids (Asvold et al., 2007a; Biondi et al., 2005; Osman et al., 2002), pulmonary system (Krude et al., 2002; Lei et al., 2003; Mendelson & Boggaram, 1991) and

kidney. Total cholesterol, low density lipoproteins (LDL), non-high density lipoproteins (non-HDL), and triglycerides increase linearly with increasing TSH, and HDL decreases consistently with increasing TSH across normal reference ranges without evidence of any threshold effect (Asvold et al., 2007b). Similar trends in lipid profiles can be identified across clinical categories from hypothyroid to euthyroid to hyperthyroid individuals (Canaris et al., 2000). Within the reference ranges for TSH, there is a linear positive association between TSH and both systolic and diastolic blood pressure (Asvold et al., 2007b). Intimal medial thickness (IMT), a measure of atherosclerosis and predictive of coronary vascular disease and stroke, is inversely related to free T_4 after controlling for lipids, clinical factors, and thyroid autoantibodies (Dullart et al., 2007). Some of these measures are ameliorated by treatment with thyroxine. Not surprisingly, deficits in thyroid homeostasis are associated with cardiovascular risk in multiple epidemiologic studies. A meta-analysis of 14 epidemiologic studies (Rodondi et al., 2006) found an overall increase in risk of coronary heart disease (CHD) of over 65% in those with subclinical hypothyroidism (elevation in TSH with normal T_4). A higher risk was noted in those studies that adjusted for most cardiovascular risk factors. Treatment with L-thyroxine of patients with subclinical hypothyroidism resulted in improvements in cardiovascular risk factors including total cholesterol and endothelial function (Razvi et al., 2007). In addition, environmental exposure to at least one thyroid disrupting chemical (PCBs) has been shown to have an inverse association with T_3 in men (Meeker, Altshu & Hauser, 2007) and was associated with both unfavorable lipid profiles and self reported cardiovascular disease in men and women (Goncharov et al., 2008). Therefore, epidemiologic as well as mechanistic and therapeutic evidence substantiates the concern that thyroid disrupting chemicals may adversely affect cardiovascular risk in humans by reducing serum T_4.

2.5.4 Evidence for endocrine disruption of the thyroid in vertebrate wildlife

Thyroid hormone is produced in all vertebrate classes and the chemistry of the hormone is identical in all of these species. In addition, thyroid hormones play a role in development in at least some members of all vertebrate classes. For example, in the flounder, metamorphosis is thyroid hormone dependent. This is also the case for amphibians. Much less is known about the capacity for thyroid dysfunction by EDCs in reptiles and in birds (with the exception of chick development, which provides an important developmental model). Thyroid hormone receptors (both TRα and TRβ) are highly conserved among the vertebrates, suggesting that thyroid disruptors in any vertebrate may exert similar effects across all vertebrate species. However, metabolism of chemicals and subsequent exposures may differ considerably among the vertebrates and there may be other important differences that would suggest that caution be used when extrapolating information from one vertebrate class to another.

Thyroid hormone disruption reported in vertebrate wildlife species includes cetaceans and other sea mammals, as well as a range of fish and birds. Some examples are given in the following sections. Effects on invertebrate wildlife have not been included: whilst thyroid hormone receptor orthologues have been reported across a range of invertebrate species, including the platyhelminths, *Schistosoma japonium* and *Schmidtea mediterranea*, the mollusc, *Lottia gigantea,* and the arthropod, *Daphnia pulex* (Wu, Niels & LoVerde, 2007), the capacity for thyrotoxic chemicals to exert effects on invertebrates is, as yet, unknown. Exposures of wildlife to thyroid hormone disrupting chemicals are comprehensively reviewed in Chapter 3.2.1.

2.5.4.1 Wild mammals

Many studies have reported relationships between individual body burdens of persistent organic pollutants and thyroid-related effects in seals (Brouwer, 1989; Hall, Kalantzi & Thomas, 2003; 2007; Routti et al., 2008), sea lions (Debier et al., 2005), beluga whales inhabiting the St. Lawrence estuary (DeGuise et al., 1995), the harbour porpoise (Schnitzler et al., 2008), and the polar bear (Braathan et al., 2004, Skaare et al., 2001), suggesting contaminant-mediated disruption of thyroid homeostasis. In some studies, interfollicular fibrosis could be seen in the thyroid gland itself, associated with severe pathological dysfunction in other animals. PDBEs and PCBs particularly affect thyroid hormone transport and metabolism (Hallgren et al., 2001; Zhou et al., 2001; Zhou et al., 2002). Thyroid hormones are described as having a permissive role in the effects of other hormones and various enzymes, are important for metabolic regulation and are necessary for adequate growth. They control some aspects of fasting and may play a role in moulting cycles (Bentley et al., 1998). They are therefore key components of the endocrine system of wild mammals and any effects on their production, secretion, metabolism and target sites will have consequences for a range of physiological processes.

2.5.4.2 Non-mammalian vertebrates

Fish in contaminated locations are known to have impaired thyroid systems. The most famous historical examples of thyroid disruption were in the salmonids living in heavily polluted regions of the Great Lakes area in the United States during the 1970s and 1980s (e.g. Leatherland & Sontesgard, 1980a; 1980b; 1982a; 1982b; reviewed in Jobling & Tyler, 2003). Moreover, in the last decade, thyroid abnormalities were also reported in mummichogs from a polluted site in New Jersey, USA (Zhou et al., 2000) and in San Francisco Bay, California, USA (Brar et al., 2010). In the latter study, plasma concentrations of T_4 were significantly reduced in two species of fish from highly contaminated areas, compared with fish from cleaner locations in the same estuary and both the T_3:T_4 ratio and T_3 concentrations were positively correlated with PCB concentrations measured in the livers of the exposed fish whilst T_4 concentrations were inversely correlated. Taken together, the results support the conclusions from laboratory experiments and the general hypothesis already indicated in some marine and terrestrial mammals that environmental PCBs may alter T_4 deiodination or turnover. Relationships between exposure to other chemicals

and thyroid hormone disruption in fish are less common, albeit increasing in the last decade, especially in relation to exposure to flame retardants (PBDEs).

In birds, biomarkers of exposure to thyroid-disrupting chemicals have also been evaluated by McNabb (2005), Panzica, Viglietti, Panzica & Ottinger (2005), and Grote et al. (2006). However, the exact extent to which EDCs exert effects on bird populations is still not established and field studies do not always support extrapolation from laboratory studies (e.g. Fernie et al., 2003; Fernie, Bortolotti & Smiths, 2003a; Fernie, Smiths & Bortolotti, 2003b), possibly because of between-species differences in susceptibility. Notwithstanding this, the relationships between the PCB concentrations and thyroid dysfunction in various bird species conducted over a long period strongly suggest that some PCBs can modulate this system in wild birds. This suggestion is now also supported by results from experimental studies on various model species. Long-term monitoring of herring gulls in the Great Lakes revealed significant thyroid dysfunction linked with PCB burden (Scanes & McNabb, 2003), and structural thyroid abnormalities detected in great cormorants from Tokyo Bay were also associated with PCDF and PCB contamination (Saita et al., 2004).

In addition, other studies on birds have found negative correlations between blood T_4 and T_4:T_3 ratio and levels of organochlorines, particularly hexachlorobenzene and oxychlordane, in glaucous gulls from the Barents Sea (Verreault et al., 2004). Similarly, reduced T_4 levels were reported in white stork nestlings exposed to pollution from a copper smelter (Kulczykowska et al., 2007). In contrast, an increase in T_3 and T_4 levels were detected within the thyroid glands of tree swallow nestlings from reclaimed wetlands partly filled with mine tailings from oil sands processing in Alberta, Canada (Gentes et al., 2007). It was postulated that the modulation of thyroid function in these birds may adversely affect metabolism, behaviour, feather development and moulting, ultimately compromising the survival of fledglings. High body burdens of PCBs in the European shag were associated with increased fluctuating wing asymmetry and also with disruption of the thyroid hormone, vitamin A (retinol) and vitamin E (tocopherol) homeostasis (Jenssen et al., 2010). Intergenerational effects of PCB exposure have also been demonstrated in kestrels, primarily via maternal transfer but also attributable to behavioural effects in the male parent. Where one or both parents had been exposed in ovo to PCBs, the progeny exhibited effects on development and growth, and sexually dimorphic effects on plasma T_3 levels (Fernie et al., 2003b).

2.5.5 Evidence for a common EDC mechanism of thyroid disruption for human and wildlife

From the above, it is apparent that many of the symptoms associated with thyroid hormone disorders in humans, namely alterations in the levels of circulating thyroid hormones and changes in the structure of the thyroid gland, have also been reported in wildlife. However, although probable, as yet there is no evidence that directly links the disruption of thyroid function via chemical exposure to adverse ecological effects in any wildlife species. In contrast, evidence of adverse effects is beginning to emerge from laboratory-based studies and will be discussed in the following section.

2.5.5.1 Evidence for EDC causation of thyroid disruption in laboratory studies with rodents and other vertebrates

Much of the laboratory-based research into the implications of EDC exposure for thyroid function in humans stems from studies using rodent models. For example, the rat has been extensively used to explore the health effects of exposure to PBDEs, with most studies consistently reporting a negative correlation with T_4 concentrations (Zhou et al., 2002; Kodavanti & Derr-Yellin, 2002; Darnerud et al., 2007). Indeed, Kuriyama et al. (2007) demonstrated that BDE-99 has the capacity to reduce T_4 levels in rats, even at low and environmentally relevant doses, with adipose tissue concentrations of BDE-99 in rats close to those reported in non-occupationally exposed humans and also at equivalent doses to those associated with other adverse outcomes in male and female rats, including permanent changes in neurobehaviour, locomotor activity and fertility (Kuriyama et al., 2005). Thus, it would appear that, in rodents, effects on thyroid function occur at EDC concentrations close to current human body burdens.

There is also laboratory-based evidence to support the assertion that EDCs are involved in the causation of thyroid disorders in wildlife species. For example, the suggestion that organochlorine pesticides, PCBs and flame retardants are causing thyroid disruption in arctic wildlife is supported by data from experimental studies on various model species such as domesticated arctic foxes, Greenland sled dogs and goats (e.g. Lyche et al., 2004; Oskam et al., 2004; Ropstad et al., 2006 Sonne et al., 2009). As a model of high trophic level carnivores, Kirkegaard et al. (2011) exposed female Greenland sled dogs and their pups to whale blubber contaminated with organohalogen compounds from 2-18 months of age and then examined thyroid hormone status. Although the sample numbers were low, the results supported observational data in other wildlife and humans, by showing that long term exposure to EDCs may result in detectable effects on thyroid hormone dynamics by lowering both free and total T_3.

In non-mammalian vertebrates, there are many laboratory studies reporting the effects of EDCs on thyroid hormone homeostasis, particularly in amphibians, due to the role of thyroid hormone in inducing metamorphosis. In this respect, BPA has been shown to block thyroid hormone-induced metamorphosis, indicating anti-thyroid activity (Iwamuro et al., 2003), which is consistent with its antagonism of T_3 binding in *Xenopus* tadpoles (Goto et al., 2006). The herbicide acetochlor was also found to accelerate T_3-induced metamorphosis of *Xenopus* (Crump et al., 2002), a process that was preceded by disruption of T_3-

dependent expression of thyroid hormone receptor genes in the tadpole tail. Nonylphenol had an overall inhibitory effect on the rate of bullfrog tadpole metamorphosis (Christensen et al., 2005). Gutleb et al. (2007) developed a synchronized amphibian metamorphosis assay, which is based on a the analysis of a range of endpoints, including the percentage of metamorphosed froglets by the end of the 60-day experimental period and the percentage of tadpoles at different stages of development, using *Xenopus laevis* as a model. Using this assay as a tool, a range of thyroid hormone disturbances were observed in response to a mixture of PCBs.

Although differences in sensitivity have been reported, depending on the model in question, in general, it would appear that the same chemicals, or groups of chemicals, elicit similar response patterns regardless of the species in question and the test system used. Laboratory-based studies using mammalian (mainly rodents) and non-mammalian species (most notably amphibians) have been invaluable in demonstrating the capacity for EDCs to affect thyroid development and in helping to identify critical periods of exposure during development. The data generated by these studies support the theory concerning the involvement of EDCs in the causation of thyroid disorders in wildlife and, in many cases, mirror the evidence concerning the etiology of these disorders in humans.

2.5.5.2 Interspecies extrapolation

Interspecies extrapolation of adverse effects of EDCs requires careful consideration. An example in which cross-species extrapolation is warranted is that of perchlorate. Perchlorate competitively inhibits iodine uptake into the thyroid gland, with subsequent decreases in TH synthesis and declines in circulating TH concentrations (Wolff, 1998). The kinetics for perchlorate inhibition of iodine uptake in humans and rats are extremely similar (US EPA, 2002), indicating the homologous nature of the initial toxic event. Although this is a clear example of a situation in which the toxic event (i.e. iodine uptake into the thyroid gland) exhibits similar kinetic profiles for rodents and humans, the impact of reduced serum thyroid hormone in rodents and humans may differ in some characteristics. For example, rodents or humans may possess robust compensatory mechanisms that would ameliorate the impacts of perchlorate exposure or low T_4 (National Research Council, 2005). However, it is not at all clear that this is the case. Studies in humans indicate that even mild iodine insufficiency is associated with lower IQ in children (Berbel et al., 2009; Zimmermann, 2007; Aghini Lombardi et al., 1995), which does not support the notion that compensatory mechanisms are robust or available to the developing brain. Moreover in animals, Gilbert & Sui (2008) found that perchlorate exposure of pregnant rats can significantly affect synaptic transmission in the adult offspring, which also indicates that robust compensatory mechanisms to low thyroid hormone are not available. In addition, Sharlin et al. (2010) failed to identify compensatory responses to low levels of thyroid hormone in the developing rodent brain.

In contrast to the above, some studies do not support direct extrapolation between species (Crofton, 2004). To illustrate this kind of situation, both in vivo and in vitro studies suggest that PCBs activate the pregnane X receptor (PXR) in rodent liver, which leads to upregulation of hepatic catabolic enzymes and subsequent declines in circulating concentrations of T_4 (Schuetz, Brimer & Schuetz, 1998). The steroid X receptor (SXR) is the human equivalent of rodent PXR (Blumberg et al., 1998) and there are species differences between these two proteins. Rodent PXR is activated by pregnenolone-16α-carbonitrile (PCN), but not by rifampicin, whereas human SXR is activated by rifampicin but not by PCN (Kliewer, Goodwin & Willson, 2002). In addition, in vitro data suggest that high concentrations of CB-153 act as an antagonist at the human SXR rather than an agonist on the PXR in rodents (Tabb et al., 2004). Thus, PCBs may cause serum T_4 to decline in animals but not in humans. While these data appear to support the conclusion that rodent data for PCBs are not relevant to humans, it does not appear to be that simple. First, if the hypothesis is correct that PCB, increase T_4 clearance in a manner similar to that of phenobarbital, then serum TSH should increase as it does in response to phenobarbital (Hood & Klaassen, 2000). Because TSH does not increase in response to PCB exposure in rodents, the mechanism(s) by which PCBs cause a reduction in serum T_4 may not be well understood. In addition, we know that some PCB congeners or metabolites can interact directly with the TR (see above), which is not related to a PXR/SXR pathway. Thus the mechanisms by which PCBs cause a reduction in serum T_4 even in animals are not fully understood, nor have the most important pathways of toxicity in animals or humans been identified. Thus, the information required to exclude animal studies for consideration in risk assessment for PCBs is not available. Moreover, there are few other chemicals for which so much information is available. Therefore, it is unlikely to be the case that animal-to-human extrapolation should be excluded.

Finally, some authors propose that there are differences in circulatory transport proteins for thyroid hormones (e.g. transthyretin and thyroid-binding globulin) in rodents compared to humans and that this renders rodents much more sensitive to thyroid hormone reducing agents than are humans (Capen, 1997; Hill et al., 1998). However, it is not clear that these differences are meaningful for two reasons. First, pregnant and neonatal rodents have high levels of all transport proteins including thyroxine binding globulin (TBG) (Savu et al., 1991; Vranckx, Savu & Nunez, 1989; Savu et al., 1989; 1987). Rat TBG has been cloned (Tani et al., 1994) and its regulation studied (Vranckx et al., 1994). Thus, the contention that rodents do not have the same serum binding proteins as humans may not be correct. A further difference between rodents and humans is that the serum half-life of T_4 in rodents is much shorter than that of humans (1 day in rodents versus 7-10 days in humans), although it is not at all clear that this issue renders rodent studies of thyroid function irrelevant to humans either; there are considerable data that suggest just the opposite.

2.5.6 Main messages

- Thyroid hormone is important in development and in adulthood in both wildlife and humans.
- Aside from thyroid cancer and congenital hypothyroidism, it is difficult to identify trends in the incidence of human thyroid disease.
- There are many chemicals that can interfere with thyroid function.
- Similarly, there are chemicals that can interfere directly with thyroid hormone action.
- Many chemicals interfere with thyroid function in a manner that will not be captured by evaluating only serum hormone levels.
- Despite the recognition that thyroid hormone is essential for brain development in humans, few if any chemicals are tested for their ability to interfere with thyroid hormone action.
- Relationships between exposure to chemicals and thyroid hormone disruption in wildlife species have increased in the last decade, especially in relation to exposure to flame retardants (PBDEs) and PCBs.
- The strength of evidence supporting a role for endocrine disrupting chemicals in disrupting thyroid function in wildlife adds credence to hypothesis that this could also occur in humans.
- Thyroid disruption is acknowledged to be poorly addressed by the chemical tests currently listed in the OECD Conceptual Framework.

2.5.7 Scientific progress since 2002

Since the *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002), the following advances have been made:

- Increasing numbers of human studies establish a link between chemical exposures and thyroid function, including in pregnant women.
- However, few studies have focused on the relationship between chemical exposures in pregnant women, thyroid measures in those women (or in the cord blood of their offspring), and cognitive function in neonates.
- Genetic lines of mice have become widely available that should be coupled with toxicology studies to help clarify the mechanisms by which chemical exposures can interfere with thyroid hormone action.
- Relationships between exposure to chemicals and thyroid hormone disruption in wildlife species have increased in the last decade, especially in relation to exposure to the flame retardants (PBDEs) and PCBs, but other chemicals are insufficently studied.

2.5.8 Strength of evidence

There is sufficient evidence that some thyroid diseases are increasing in the human population and that this baybe related to environmental exposures. These diseases include congenital hypothyroidism and thyroid cancer. This evidence is considered to be sufficient because several authors report an increased incidence using screening data that reflect population-wide surveys. However, there are insufficent data linking these increases in thyroid disease to specific environmental factors.

There is limited evidence from wildlife studies and sufficient evidence from laboratory experiments that endocrine disrupting chemicals can interfere with thyroid hormone signalling, leading to diseases and disorders in wildlife species. The data generated by these studies support the theory concerning the involvement of EDCs in the causation of thyroid disorders in wildlife and mirror some of the evidence seen in humans. For many wildlife species, however, no studies have been done.

There is insufficient direct evidence in the human literature supporting the hypothesis that effects on thyroid hormone signalling mediate the association between chemical exposures and human disease/disorders. Perhaps the best example of this is focused on PCBs. There is sufficient evidence linking PCB body burden to reduced measures of cognitive function in children (Schantz, Widholm & Rice, 2003) and this evidence is deemed to be sufficient because a number of authors have reported similar findings and because it is consistent with studies in animals. In animal studies, PCBs clearly reduce circulating levels of thyroid hormone (Brouwer et al., 1998) and can affect brain development (Roegge et al., 2006). There are some studies indicating that PCB body burden is linked to reduced measures of cognitive function, but the evidence demonstrating a causal relationship is limited. Few studies have evaluated the relationship between PCB exposure, cognitive development, and thyroid hormone; therefore, there is overall insufficient evidence to demonstrate that PCBs interfere with thyroid hormone signalling and cause an adverse effect. Animal studies indicate that PCBs can exert effects on thyroid hormone signalling in development that are not consistent with effects on serum hormone levels (Bansal & Zoeller, 2008; Giera et al., 2011). Therefore, while considerable evidence exists in animal studies that chemicals can interfere with thyroid hormone signalling during development and produce adverse outcome, we have not developed the approach to fully test this hypothesis in human populations.

Thus, there are insufficient data linking chemical exposures to altered thyroid hormone signalling and the occurrence of disease or dysfunction in humans. Clearly, considering the importance of thyroid hormone during development, the large knowledge gaps, animal data, and the economic cost of population wide impacts on thyroid function during development (Dosiou et al., 2008), these are issues that need to be addressed quickly.

2.5.9 References

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2.6 Endocrine disruptors and neurodevelopment in children and wildlife

2.6.1 Overview of neurodevelopmental problems in humans and wildlife and evidence for endocrine disruption

There is currently considerable concern about a potential relationship between increasing prevalence of neurodevelopmental disorders and the increase in exposure to pollutants over the past several decades (Landrigan & Goldman, 2011a; 2011b; Weiss & Landrigan, 2000). Since the 1970s, there have been dramatic increases in previously rare neurodevelopmental disorders. For example, in the 1970s autism prevalence was estimated to be between 4 and 5 in 10,000 children (Wing et al., 1976) but today this value is estimated to be 1 in 110 children (Rice, 2007). Similar trends have been observed for other neurobehavioural problems such as ADHD (attention deficit hyperactivity disorder) and autistic disorder (**Figures 2.12** and **2.13**), learning disabilities and childhood and adult depressive disorders. Predominant among these disorders are attention deficit disorders (ADD) – with or without hyperactivity – with a worldwide pooled prevalence estimate of about 5.3% (Polanczyk et al., 2007). Whilst the increase in autism spectrum disorders is indisputable, questions remain as to whether the increase in the incidence and prevalence of ADHD represent a true increase rather than an artefact due to more agressive disagnosis and reporting. There are also questions regarding whether there are biological determinants of ADHD that may be impacted by the environment. There are brain imaging studies that support the concept that there are biological differences between children with ADD compared to those children without ADD (Aguiar, Eubig & Schantz, 2010). In addition, genetic studies show a link between ADHD and genotype, though this is modified by environment (Khan & Faraone, 2006). Therefore, it remains a significant challenge to identify the possible causes of the increased incidence – either geographical or temporal – and to determine the extent to which environmental factors play a role (Aguiar, Eubig & Schantz, 2010).

The observation of Paracelsus that women with goitre gave birth to children with severe mental retardation was an early indication that environmental factors could affect brain development and neurobehaviour (Cranefield & Federn, 1963). Likewise, lead poisoning has been known to cause neurotoxicity for millennia, though this was believed to be a disease of adults working in occupations in which lead exposure was very high (Needleman, 2009). Since then, our knowledge of the relationship between neurodevelopmental disorders and chemical exposure has advanced. It is now clear that children – especially during fetal development – are sensitive to the neurotoxic effects of lead and mercury, and at low levels (e.g. Needleman, 2009). It is less widely appreciated that hormones play many critical roles in neurodevelopment and, therefore, associations between chemical exposures and neurobehavioral disorders in humans and wildlife could be plausibly related to disruptions of endocrine pathways. Perturbations in thyroid hormone homeostasis during early life can alter the neuroendocrine circuits that co-ordinate sex-

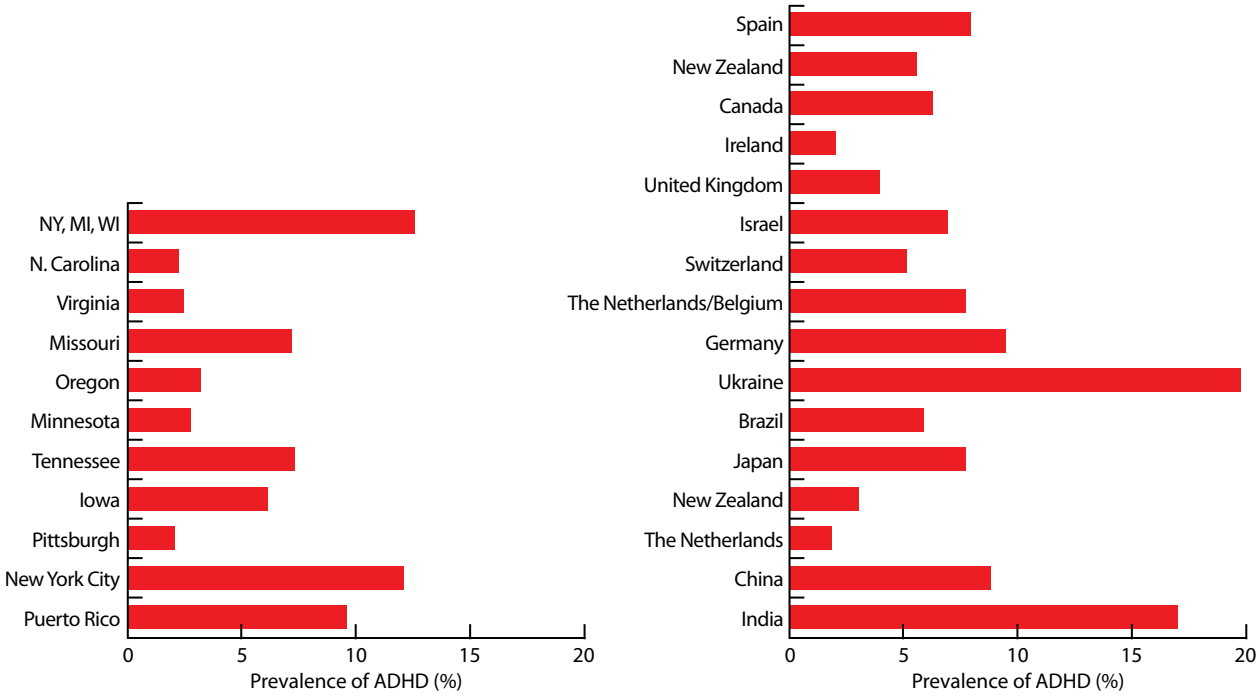

Figure 2.12. Worldwide prevalence of ADHD in children (http://www.medscape.org/viewarticle/547415). Figure reproduced with publisher's permission.

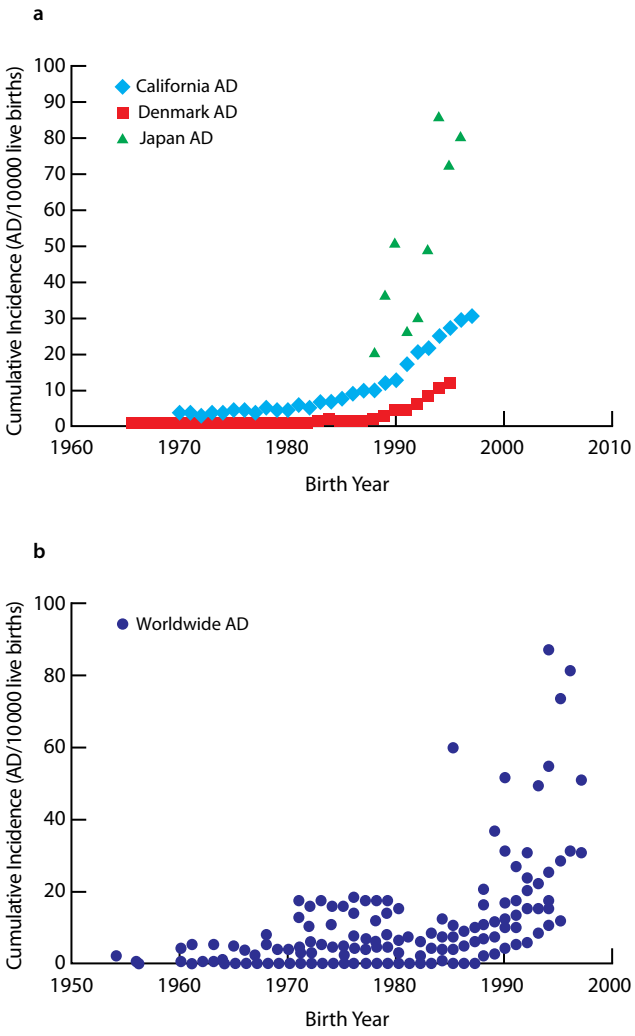

Figure 2.13. Autistic disorder (AD) cumulative incidence time series by cohort birth year from the literature for (a) Denmark, California, and Kohoku Ward, Japan and (b) worldwide AD cumulative incidence. Figure taken from: Timing of Increased Autistic Disorder Cumulative Incidence. (Figure from McDonald & Paul (2010), redrawn; Used with publisher's permission)

specific physiology and behaviour (Jugan, Levi & Bloneau, 2010) and lead to a series of psychiatric and behavioural conditions that are becoming increasingly evident in our society (Gore, 2008, Mclachlan, 2001). The reproductive hormones – estrogens, androgens, progestins – likewise have important effects on neurodevelopment and childhood behaviour, as well as effects on the brain of adults and adult disease. Receptors of these hormones are expressed in the developing brain – in some cases under regulation by other hormones - indicating the great number of interactions hormone systems have on the developing brain. Not only are these hormones involved in the development of sex-typical behaviours – critical attributes in wildlife populations – but they are also involved in the development of other brain structures.

Evidence suggests that endocrine disrupting chemicals can interfere with neurodevelopment affecting cognition and sexual behaviour in both wildlife and humans:

- **There are sufficient data in human populations to conclude that exposures to PCBs during fetal development are linked to general cognitive deficits (e.g., lower global intelligence quotient).** Even in studies of relatively low exposures, PCBs are correlated with measures of cognitive function.
- **Alterations in sexually dimorphic behaviours are seen in human populations highly exposed to PCBs.**
- **Limited data exist to show that *in utero* exposure to other EDCs also affects cognition and sexually dimorphic behaviours in animal studies.**
- **Recent studies of aquatic birds and fish suggest that methylmercury exposure at environmentally relevant levels can interfere with reproductive success** due either to overt neurotoxicity or more subtle neuroendocrine disruptive effects. Methylmercury-exposed birds in the field and in the laboratory have shown altered testosterone and estradiol concentrations (Frederick & Jayasena, 2011), as well as altered courtship behaviour, altered song (Hallinger et al., 2010), high levels of male-male pairing and reduced reproductive success (Frederick & Jayasena, 2011). Reproductive behaviours are also affected in fish exposed to environmentally relevant concentrations of methylmercury (Hammerschmidt et al., 2002; Sandheinrich & Miller, 2006), likely due to its effects on the endocrine system (Crump & Trudeau, 2009).
- **Many areas of the world are still inhabited by wild mammals with levels of methyl-mercury in their tissues that would be unsafe for rodents and humans** (Basu & Head, 2010; Mergler et al., 2007).

2.6.1.1 Thyroid hormone insufficiency and brain development

The neurobehavioural impacts of thyroid hormone insufficiency in humans are so clear that there is universal screening of thyroid function in all regions of the world (LaFranchi, 2010). To understand the ways in which exposure to endocrine disruptors could affect brain development, it is necessary to understand the complexities of the development of both the neurologic and thyroid systems and how thyroid hormones regulate brain development.

Figure 2.14 illustrates the three stages of neurological development in relation to thyroid hormone during fetal development, thus highlighting why the fetus is sensitive to thyroid hormone disruption (Williams, 2008). There are three stages of thyroid hormone-dependent neurological development depicted in the figure: the first is before the onset of fetal thyroid hormone synthesis (16-20 weeks post conception in humans), the second is during the rest of pregnancy, when the developing brain derives thyroid hormones from both the mother and the fetus, and the third is in the neonatal and post-natal period when thyroid hormone supplies are derived from the child. Thyroid hormone plays

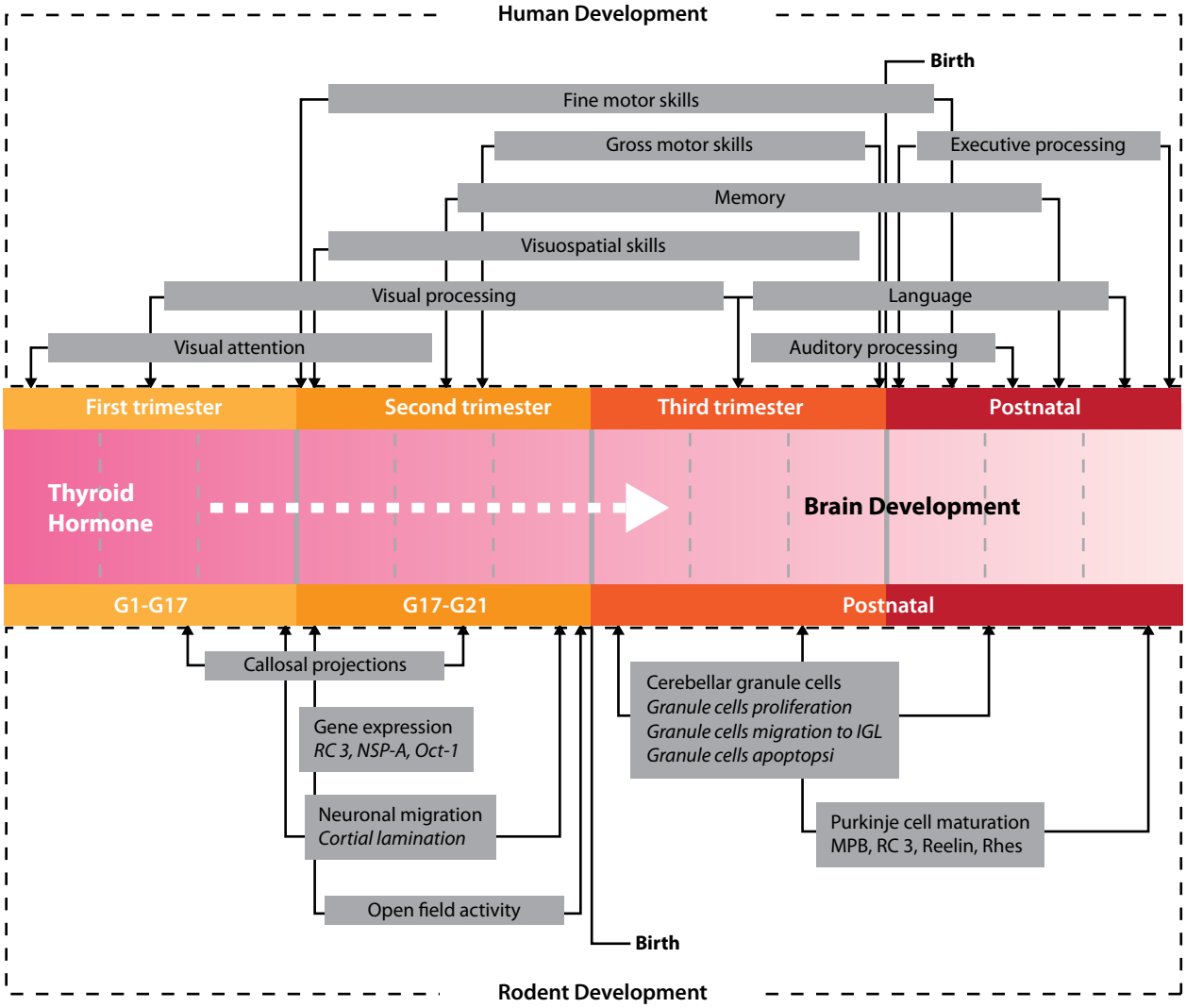

Figure 2.14. Relationship between thyroid hormone action and development of the brain. In the first trimester of pregnancy, early neuronal proliferation and migration is dependent on maternal thyroxine (T4). By the end of the first trimester, development of the hypothalamic-pituitary axis has occurred and a surge in thyroid-stimulating hormone (TSH) secretion results in the onset of fetal thyroid hormone production and increasing occupation of thyroid hormone receptors (TRs) by T3. Continuing development of the brain in the second and third trimesters relies increasingly on T4 produced by both the fetus and mother. Continued post-natal development is entirely dependent on neonatal thyroid hormone production (Figure based on Zoeller & Rovet, 2004).

different roles in different parts of the brain at various times during development (Zoeller & Rovet, 2004). It is delivered to the brain in a complex shuttling system. Largely, T_4 is transported across the blood brain barrier by specific transporters, converted to T_3 in the supporting (glial) cells, and then further transported to neurons (Bernal, 2005). Thus, it is a combination of transporters and enzymes that regulate the delivery of the hormonally active T_3 to its targets. These targets are both glial cells and neurons. For example, the cells that produce the insulating sheath (myelin) around the axons in the brain are dependent upon thyroid hormone (Billon et al., 2002) and animals with low thyroid hormone show progressively fewer of these cells in the major bridge between the two hemispheres of the brain (Sharlin et al., 2008). Genetic defects of the T_3 transporter in humans cause several mental deficits to occur (Maranduba et al., 2006; Schwartz et al., 2005; Visser et al., 2009).

2.6.1.2 The role of hormones in brain development

Sex steroids

In addition to the changes in thyroid hormone exposure during early development, both male and female rodent embryos are exposed to a changing milieu of sex steroid hormones during the late embryonic and early postnatal period that cause permanent sexually dimorphic differences in the size, cell number and neurochemistry of hypothalamic regions of the brain (reviewed in McCarthy, 2009). Because male and female embryos are exposed to different genetic and hormonal environments, the brains of male and female newborns are substantially different from the day of birth. The existence of a genetic contribution to the control of sexual dimorphisms in neurobiology and sexual orientation is firmly established, although the specific genes implicated in the process are not known. The role of hormones in human sexual

orientation is less well understood. Balthazart (2011) reviews the literature indicating that sex steroid hormones may act in concert with genetic factors as well as features of the social postnatal environment to influence sexual orientation.

Balthazart (2011) reviews the literature indicating that sex steroid hormones may act in concert with genetic factors - and features of the postnatal social environment - to influence sexual orientation in humans. This review emphasizes the heuristic value of studies of girls with a medical condition known as congenital adrenal hyperplasia (CAH). These girls are exposed in utero to high levels of androgens from the adrenal glands and exhibit, as a population, masculinization of various behaviors including aggressive play and increased probability of homosexual relationships (30-40 % in some studies compared to 10% in case controls or unaffected sisters) (Balthazart, 2011).

Other morphological and physiological characteristics also appear to be influenced by prenatal testosterone in CAH women, such as the ratio of the lengths of the second to fourth fingers which differs between males and females (Hampson, Ellis & Tenk, 2008) and is clearly masculinized in these women (Breedlove, 2010).

In rodent models of human physiology, studies show that sex differences in brain structure are caused by differences in hormone action early in fetal development. These structural differences are irreversible and are parallel to the effects of hormones early in development on adult sex behavior. Thus, hormones act early in development to organize the nervous system in such a way that hormones in the adult can activate sex-typical behaviours. In rodents, exposure to the male hormone testosterone induces the preference for a feminine sexual partner. Thus, genetic males or genetic females exposed to testosterone will orient toward a female. In contrast, the absence of testosterone leads to preference for a masculine partner; genetic males or females deprived of testosterone during development will orient toward a normal male. It is also important to recognize that testosterone is converted to estrogen in the male brain, and it is estrogen that is responsible for sexual orientation (Henley, Nunez & Clemens, 2009; 2011).

Hypothalamic and pituitary hormones

Multiple hypothalamic neuropeptides and neurotransmitters as well as pituitary hormones exert control over sexual behaviour and reproduction in vertebrate animals including humans (reviewed in Dickerson & Gore, 2007). The reproductive neuroendocrine axis in vertebrates is regulated by the gonadotropin releasing hormone (GnRH) neurosecretory system, located at the base of the hypothalamus. In the pituitary, GnRH binds to its receptors and stimulates the synthesis and release of the gonadotrophic hormones LH and FSH into the general circulation. The gonadotrophins act at the gonads (ovaries and testes) to stimulate sex steroid production, gonadal maturation and sperm and egg production. These three levels of the hypothalamic, pituitary gonadal axis function both independently and interdependently and thus a dysfunction at one level has consequences for the other levels. Moreover, neurotransmitters also modulate the release of GnRH.

Each neurotransmitter may have more than one type of receptor on more than one type of cell and therefore, alterations in the level of a single neurotransmitter may affect multiple cells in different ways. Chemical contaminants can affect both neurotransmission and neurosecretion via various mechanisms. Minor changes in neuronal function may cause major changes in sexual behaviour.

2.6.2 Evidence for endocrine disruption of neurodevelopment in children and in rodent models

2.6.2.1 Attention deficit disorders

There are a number of studies that support the hypothesis that specific environmental factors represent risk factors for ADD. Lead and PCB exposures represent important cases of environmental contaminants associated with ADD in children (Eubig, Aguiar & Schantz, 2010). Exposure to lead is particularly high in developing countries like in many parts of Africa where more than one third of the children still suffer high levels of lead exposure (Falk, 2003). In developed countries, on the other hand, only a small minority of children (mainly the urban poor) are still affected by high levels of lead (reviewed in WHO, 2003). Likewise, attention deficit is over represented in children whose mothers exhibited low thyroid hormone in the first trimester of pregnancy (Haddow et al., 1999) or in children with prenatal ethanol exposure (Mattson, Crocker & Nguyen, 2011). Finally ADD has been linked to elevated exposure to a variety of organophosphate pesticides (Bouchard et al., 2010; Kuehn 2010; Marks et al., 2010; Riccio, Avila & Ash, 2010; Schettler 2001; Xu et al., 2011) still found in relatively large segments of human populations (see Chapter 3.1.1.6 & 3.2.2.2). Thus, overall, although there is uncertainty about causes of the increased incidence and prevalence of ADD in children worldwide, there is plausible evidence to conclude that some environmental chemicals are associated with this disorder.

2.6.2.2 General cognitive deficits and PCB exposure

A very large number of studies have been published over the past 10 years designed to characterize exposures of children to industrial chemicals and to test whether they are related to measures of cognitive deficits in children. In particular, the relationship between exposures to PCBs and measures of cognitive function has been well-studied. Despite the fact that PCB production was banned in the late 1970s, they are still found in all environments and all human and animal tissues (see Chapter 3 for a comprehensive review). The relationship between PCB exposure and cognitive function is an important topic (reviewed by Carpenter, 2006) for the following reasons: 1) a large number of high quality studies have been published on human populations around the world, 2) exposure assessment has become quite sophisticated, and 3) cell-free,

cell-based, and animal studies provide important insights into the mechanisms by which these and other endocrine disrupting chemicals can produce neurotoxic effects.

There are sufficient data in human populations to conclude that exposures to PCBs during fetal development are linked to general cognitive deficits (e.g., lower global intelligence quotient). In highly exposed populations, the most consistent effects across all studies were impaired executive functioning, followed by processing speed, verbal ability and visual recognition memory. These populations include: the Yu-Cheng children in Taiwan (born to mothers exposed to thermally degraded PCBs between 1978 and 1979; Chen et al., 1992), the Dutch cohort (Patandin et al., 1999), the Lake Michigan cohort of children born to mothers who ate PCB contaminated fish (Jacobson & Jacobson 1996; 2003), the Dusseldorf cohort (Walkowiak et al., 2001; Winneke et al., 1998) and the Slovakian cohort (Park et al., 2009). Moreover, even in studies of relatively low exposures, PCBs are correlated with measures of cognitive function, including impulse control (Stewart et al., 1999; 2000; 2003a; 2003b; 2005; 2000; 2008; 2006).

In addition to the cognitive deficits observed, the Yu-Cheng children also exhibited alterations in sexually dimorphic behaviour with exposed boys having a deficit in spatial abilities (Guo et al., 2004). Exposure to higher ambient levels of PCBs has also been associated with less masculinized play in boys and more masculinized play in girls in a group of Dutch school children (Vreugdenhil et al., 2004).

PCB levels detected in blood today are markedly lower than they were in the 1970s – 1990s and a study carried out in Germany recently suggested that exposure to PCBs at current exposure levels does not impair neurodevelopment. This conclusion was based on studying two populations in close proximity to each other in Germany (Wilhelm et al., 2008). Taken together, the available epidemiological evidence is sufficient to conclude that PCB exposures during fetal development are linked to measures of cognitive deficits (Schantz, Widholm & Rice, 2003).

2.6.2.3 Animal studies with PCBs

The mechanism(s) by which PCBs produce developmental neurotoxic effects have been studied extensively. A dominant theory is that PCBs can interfere with thyroid hormone signalling during development. Many of the cognitive deficits linked to PCB exposures are similar to those associated with pre- and post-natal thyroid hormone insufficiency (Zoeller and Rovet, 2004). Rodent studies almost uniformly show that PCB exposures decrease serum thyroid hormone levels (Bastomsky 1974; Goldey et al., 1995; Zoeller, Dowling & Vas, 2000) and produce effects on brain development that are similar to those seen in PCB-exposed human populations (Goldey & Crofton, 1998; Goldey et al., 1995; Herr, Goldey & Crofton, 1996). Cell-based studies show that some PCB congeners can interfere directly with the thyroid hormone receptor (Gauger et al., 2007; Iwasaki et al., 2002; Koibuchi & Iwasaki, 2006; Miyazaki et al., 2004; Miyazaki et al., 2008).

Even in rodent animal models of humans, however, it is difficult to say with certainty that behavioural or developmental effects of PCB exposure are caused directly by effects on thyroid hormone signalling. Specific PCB congeners can affect the intracellular regulation of calcium in rodent brain that is very important in nerve cell development and function (Pessah, Cherednichenko & Lein, 2010). They can also influence neurogenesis, neuron proliferation and differentiation (Fox et al., 2010), and the dopaminergic system, in vitro and in vivo (Barkley 1998; Kirley et al., 2002; DiMaio, Grizenko & Joober, 2003), thought to be crucial for the pathogenesis of ADHD. Very low doses of PCBs can impact sex steroid-related endpoints in the rodent brain (Dickerson et al., 2011a; Dickerson, Cunningham & Gore, 2011). Therefore, it is not possible to directly demonstrate in animals that PCBs produce neurotoxic effects by acting on thyroid hormone signalling alone or whether in combination with other mechanisms.

Considering this, it is even more difficult to prove that thyroid disruption mediates the effect of PCB exposure on developmental neurotoxicity in humans. A number of studies have evaluated the relationship between serum thyroid hormone and PCB body burden in humans (Miller et al., 2009; Longnecker, 2000). These are challenging studies to review both because the technology associated with PCB measurement has changed over the years, and because there are different measures of thyroid function that have been employed in these studies – as well as differences in the timing of sample collection relative to periods of exposure. In addition, different PCB congeners have different potencies for reducing thyroid hormone levels in rodents (e.g. Giera et al., 2011), and this needs to be considered also in epidemiological studies (Chevrier et al., 2007).

There are several important lessons from the PCB story:

- The only clinical measure of thyroid disruption currently available in humans is serum hormone levels, and therefore it is not currently possible to demonstrate that an association between chemical exposure and hormone level mediates specific adverse effects.
- Thyroid hormone insufficiency produces different effects on cognitive development when it occurs at different times during development (Zoeller & Rovet, 2004). Therefore, the timing of measurements of thyroid function and chemical exposure and the cognitive domains that may be affected by these exposures are critical.
- If chemicals can interfere with thyroid hormone action in a manner that is not revealed by changes in thyroid hormone levels, (as has been shown in animal studies, e.g. Giera et al., 2011), then we currently have no way of testing for this in human studies. Therefore, biomarkers of thyroid hormone action should be developed both for use in the clinic and epidemiological studies.
- No guideline study validated for use in screening or testing evaluates measures of thyroid hormone action; therefore, these chemicals would be missed by regulatory tests designed to screen chemical safety.

2.6.2.4 PBDEs and cognitive disorders

Knowledge of developmental toxicity of PBDEs is limited, although human in vitro and epidemiological studies indicate that they work through the same mechanisms as PCBs to induce effects on neurodevelopment via thyroid hormone disruption (Schreiber et al., 2010; Chevrier et al., 2010). Johnson-Restrepo & Kannan (2009) determined that infant daily exposure dose of PBDEs in the USA due to inhalation, incidental oral ingestion and dermal absorption of house dust were significantly higher than in adults. Moreover, serum samples of infants aged 0-4 years contained significantly higher PBDE concentrations as compared to children of 5-15 years of age in an Australian population (Toms et al., 2008). The major exposure route to PBDEs, however, is through maternal exposure in breast milk (reviewed in Chapter 3.2).

There are few epidemiological studies on the neurodevelopmental effects of PBDEs. A single study of 329 mothers in lower Manhattan, New York, examined 210 cord blood samples for PBDEs and neurodevelopmental effects in the children at 12-48 and 72 months of age. The findings indicated associations between high concentrations of BDE-47, -99 and -100 and lower psychomotor and mental development and IQ (Herbstman et al., 2010). An earlier study (Roze et al., 2009) also reported a similar association.

2.6.2.5 Animal studies with PBDEs

In vivo experimental studies show that maternal exposure of rodent models to individual PBDE congeners or commercial pentabrominated mixtures causes dramatic changes in thyroid hormone levels (Darnerud et al., 2007; Kodavanti et al., 2010) as well as subtle changes in neurobehaviour and both male and female reproductive endpoints (Kodavanti et al., 2010). Various studies also report long lasting hyperactivity and reduced performance in learning and memory tests (e.g. Branchi et al., 2003; 2005; Viberg 2009a; 2009b; Viberg, Fredriksson & Eriksson, 2003; 2004; Viberg, Mundy & Eriksson, 2008; Kuriyama et al., 2007; Hallgren & Darnerud, 2002; Zhou et al., 2002) in a similar fashion to that described for PCBs. Moreover, feminization of sex-steroid dependent behaviour, such as of the sexually dimorphic sweet preference of male rats, was observed following prenatal exposure to BDE-99 (Lilienethal et al., 2006) and to a PCB mixture resembling that found in human breast milk. In both of these cases, the effects on behaviour appeared to coincide with decreasing aromatase activity in the hypothalamic/pre-optic area of the brain, inhibiting the local production of the hormone estradiol (one of the main processes by which the brain becomes male-like). Parallel to these behavioural changes, alterations in proteins involved in neuronal survival, growth and synaptogenesis are seen (Dingemans, Van den Berg & Westerink, 2011). It is important to note that behavioural toxicity in rodents can also occur without alternations in maternal serum T4 (Gee & Moser, 2008; Gee, Hedge & Moser, 2008).

2.6.2.6 Mercury and neurodevelopment

Metals such as lead and mercury can also impair neurodevelopment through direct neurotoxic effects, through effects on thyroid function or through epigenetic mechanisms (Ellingsen et al., 2000; Takser et al., 2005). Methylmercury induced disruption of GABAergic signalling in the brain under probable and relevant exposure scenarios can have profound consequences as GABA (A) is the main inhibitory neurotransmitter in the mammalian brain, accounting for 50% of synapses in certain brain regions. Exposure to methylmercury results in build-up of GABA (A) neurotransmitter levels in the synapse and a corresponding decrease in GABA (A) receptor levels (Basu et al., 2010). It also reduces the availability of selenium which is essential for deiodinase activity that in turn activates and inactivates thyroid hormone in the brain and other tissues.

There is a particular current concern about methylmercury because of its high levels in the diet (Trasande et al., 2006; see Chapter 3.1.3). Historical incidences of methylmercury poisoning led to neurodevelopmental impairments in prenatally exposed children.

Consumption of fish is the primary route via which humans are exposed to methylmercury and it is estimated that 8-16% of USA newborns have cord blood levels higher than acceptable limits (Trasande, Landrigan & Schechter, 2005), although this percentage is higher in populations in all parts of the world that rely more heavily on fish for sustenance (Hightower, O'Hare & Hernandez, 2006), particularly in developing countries where fish-eating communities may be exposed to pollution from mercury processing plants (Oosthuizen & Ehrlich, 2001).

In West Greenland, for example, the levels of mercury in the human diet exceed acceptable tolerable daily levels by 50%, much of which comes from consumption of seal tissues (Johansen et al., 2004). The median concentration in the human brain of 17 Greenlanders was 0.17 µg/g wet weight, although levels of 4mg/g were found in some humans (Pedersen et al., 1999). Furthermore, a study of 43 Inuit children reported that mercury exposure might be related to neurological deficits (Weihe et al., 2002).

In Africa, Nweke & Sanders (2009) report that an important source of direct mercury exposure is the artisanal gold mining and processing when exposure to vaporized elemental mercury occurs during burning to separate the gold-mercury amalgam (Savornin, Niang & Diouf, 2007). Workers typically not equipped with personal protective equipment are at risk as well as children under their care (Van Straaten, 2000). In some countries, the mining and sale of gold are a female-only activity and this may include a workforce between 500 and >100,000 women and children (Hentschel, Hruschka & Priester, 2003).

2.6.2.7 Bisphenol A and phthalates may affect sex-specific behaviours and sex dimorphism in neural development

Yolton et al. (2011) recently showed that the concentrations of BPA and phthalates in maternal urine during early pregnancy

were associated with higher hyperactivity and aggression in 2 yr old girls, but not in boys, consistent with rodent data, suggesting an effect of BPA on sexual dimorphism of these types of behaviour, (Kubo et al., 2003, Rubin et al., 2006). In a further study, juvenile female rats exposed to BPA during gestation and lactation exhibited defeminization of social interactions, including reduced play with males, decreased social grooming, increased play with females and increased sociosexual exploration (Farabollinii, 2002, Porrini et al., 2005). Males exhibited increased aggression at sexual maturation (Kawai et al., 2003) and increased anxiety-related behaviour.

The effects of BPA on the brain and behaviour are assumed to be attributed to its estrogen receptor (ER)-mediated action, but it is not clear how its low potency could account for the strong effects that are observed in many tissues after exposure to relatively low doses. There is also evidence that changes in gene expression in utero persist into adulthood (e.g. Smith & Taylor, 2007; also reviewed by WHO, 2011) and hence possibly involve epigenetic mechanisms (see Chapter 1.3.6). This is supported by evidence that estrogen and some endocrine disruptors have been reported to dynamically change the methylation status of their target genes and that this is of critical importance for the function of the central nervous system; epigenetic mechanisms play a crucial role in neuronal plasticity (Borrelli et al., 2008) and thus are potential targets for neurodevelopmental effects of chemicals that induce cognitive dysfunction in human populations, mainly when the exposure takes place during prenatal and early postnatal development (Vahter, 2008; Bellinger, 2008). Given the widespread use and human exposure to chemicals such as phthalates and BPA (reviewed in Chapter 3.2), this is an important area for further study.

2.6.2.8 Are mixtures of different neuroendocrine disruptors a concern for human health?

There is almost no information concerning the effects of mixtures of neuroendocrine disruptors, even though there is little doubt that PBDEs, PCBs, mercury and several pesticides will co-occur in human tissues. Examples of situations where interactive effects of mixtures been suggested to occur include the combination of methylmercury and PCBs in two large cohorts of children in the Faroe islands (Grandjean et al., 2001; 2004; Roegge et al., 2004). These suggestions are supported by a very limited number of rodent studies in which synergistic changes in neurochemical measures (e.g. Bemis & Seegal, 1999) and increases in neurotoxic effects (Eriksson et al., 2003) have been reported as a result of combined exposures to PCBs and methylmercury.

2.6.3 Evidence for endocrine disruption of neurodevelopment in wildlife

In comparison with the evidence of neurodevelopmental diseases and disorders in humans, data describing patterns of neuroendocrine dysfunction in wildlife are less prevalent, despite the fact that studies on wildlife (particularly mammalian wildlife) can provide important information on environmental exposures, early and sub-clinical effects and clinical neurotoxicity of chemicals in the environment. There is, however, a wealth of literature on the neurotoxicology of mercury, the pesticide DDT, PCBs and PBDEs. Some case studies are highlighted here that add weight to the evidence presented in the human health section of this review on the environmental contaminants of neurotoxic concern to humans.

2.6.3.1 Mammals

Methylmercury, PCBs and PBDEs biomagnify (concentrate) up through aquatic food webs, resulting in high concentrations in fish and other top predators (Chapter 3.1.3 & 3.2). As such, consumption of contaminated fish represents the primary route via which wildlife and humans are exposed to these chemicals.

Methylmercury

Much of our knowledge concerning neurotoxicology of methylmercury was obtained following the human poisoning event in Minimata Bay, Japan, alerted 5 years earlier by the frenzied behaviour of cats, rats, crows and fish (Aronson, 2005). Around this time, population declines in other wildlife species were particularly noticeable in regions that used organomercurial fungicides, or that were located downstream of pulp and paper mills using mercury.

The structural brain lesions and effects of methylmercury are similar across mammals (reviewed in Basu & Head, 2010).The organic form of mercury, methylmercury, crosses the blood-brain barrier and can cause a range of effects on brain tissues in vertebrate wildlife. Lower exposures reduce the levels of key enzymes (cholinesterase and monoamine oxidase) in wild otters (Basu et al., 2007a) and *N*-methyl-D-aspartate (NMDA) glutamate receptors in wild mink (Basu et al., 2007a; 2007b), bald eagles (Rutkiewicz et al., 2011) and polar bears (Basu et al., 2009). These effects have been corroborated in laboratory studies of mammals and fish exposed to methylmercury (Basu et al., 2006; 2007c; 2010; Coccini et al., 2006; Berntssen, Aatland & Handy, 2003) and are of both ecological and physiological concern because these enzymes and receptors are parts of critical neurochemical pathways that control reproduction, cognition, growth and development (Manzo et al., 2001). At the present time, overt episodes of mercury poisoning are rare but there is evidence that lower levels of exposure can affect growth, reproduction and development in wildlife. These effects are much more common in longer-lived species that are higher up in the food web because of the biomagnification of methylmercury through aquatic systems (see Chapter 3.1.3). It is entirely possible that even subtle neurological damage in fish-eating wildlife may be having more severe consequences than we can currently ascertain.

Mammalian wildlife species also accumulate mercury in their brains where it can have subtle effects on the brain neurochemistry (Manzo et al., 2001; Scheuhammer et al.,

2007). Many areas of the world are still inhabited by wild mammals with levels of methylmercury in their tissues that would be unsafe for rodents and humans (Basu & Head, 2010; Mergler et al., 2007). However, it is important to note that there are differences in susceptibility between different species of mammals. For example, levels of mercury in the livers of polar bears in the Canadian Arctic exceeded those in the livers of humans that succumbed to Minimata disease, but there is little observational or experimental evidence of neurological damage in the brains of these bears (Sonne et al., 2007), probably because the levels in the brain stem were markedly lower than in the other tissues of the body (Basu et al., 2009). Notwithstanding this, mercury associated changes in brain NDMA receptor levels were found in these bears, one of the earliest known responses to mercury exposure. In addition, a subsequent study reported an inverse association between mercury exposure and DNA methylation in the lower brain stem of male (but not female) polar bears, suggesting possible long term consequences of mercury exposure for chromosomal stability, disease progression and reproductive function (Pilsner et al., 2010). These results may be of relevance to human health in Greenland as an epidemiological study of 43 Inuit children in Greenland reported that mercury exposure in humans might be related to neurological deficits (Weihe et al., 2002).

PCBs

Most PCBs, particularly those with non-coplanar structures, have intrinsic neurotoxic properties (Mariussen & Fonnum, 2006), and can impede several neurological processes including dopaminergic signalling and calcium homeostasis. Whilst their action on the neurological system is clear, data regarding their accumulation in the brain are sparse. Where these data exist, liver to brain ratios range between 3-fold to more than 7-fold across mammals alone, making it difficult to derive exposure-response relationships (Giesy & Kannan, 1998; Kodavanti et al., 1998). As with mercury, the initial discoveries concerning neurotoxicological effects of PCBs were seen in wildlife. Several PCB mixtures and individual congeners at environmentally relevant levels (e.g. <1µg/g in the diet) could impair numerous health aspects including neuroendocrine function (reviewed in Basu et al., 2007b). PCB bans and restrictions have led to a decline in PCB concentrations in humans and wildlife over the past few decades, although geographic hotspots still exist where certain PCB congeners persist (Chapter 3.2.1 & 3.2.2). A few biomonitoring studies report PCBs in the brain tissues of mammalian wildlife and humans between 2-50 ng/g wet weight. In marine mammals, however, brain PCB levels are higher (up to 450 ng/g wet weight). Dominant congeners in the brain of mammalian wildlife are coplanar and are similar to those found in humans (CB153, 180, 170/190, 138 and 99).

As in humans and rodent models, the most commonly observed effects of PCB exposure is the disruption of thyroid hormone homeostasis. Laboratory studies with mink and with harbour seals have shown PCBs to decrease T3 and T4 (see Chapter 2.5). Moreover in numerous field studies of seals, sea lions and polar bears, decreased serum T4 was correlated with PCB exposure. There is, however, mixed evidence on the impacts of PCBs on brain neurochemistry in mammalian wildlife. In river otters, no significant correlations between brain PCB levels and several neurochemical markers were found (Basu et al., 2007c), whereas in captive female mink and in rodents and monkeys, changes in dopamine levels in the brain and hypothalamus were found following exposure to PCBs (reviewed in Seegal, 1996).

PBDEs

As already mentioned, the levels of PBDEs in the environment rapidly increased with the increasing popularity of PBDEs as flame retardants (Chapter 3.2). In the Baltic Sea, atmospheric deposition of PBDE still exceeds PCBs by a factor of 40X. Between 1981 and 2000, levels of PBDEs in the blubber of Arctic ringed seals and in the marine mammals of the temperate Asia-Pacific region increased about 9-fold (Ikonomou, Rayne & Addison, 2002; Tanabe et al., 2008). A single study reports levels of PBDEs in river otter brain at concentrations ranging from 1.1 to 6.6 ng/g wet weight, comprising only BDEs -99, -100 and -153 (Basu, Scheuhammer & O'Brien, 2007). Levels in avian species in Belgium are reported to be much higher (Voorspoels et al., 2006b): wild sparrows, 140-5800 ng/g; owls, 0.8-174 ng/g; and buzzards 0.2-1600 ng/g. Recent analyses of wildlife and human tissues for PBDEs show some declining concentrations due to restrictions and bans on their use, but levels in wildlife remain highest near urban centres (Voorspoels et al., 2006a) and vary considerably from one country to another as for humans (Chapter 3, sections 3.2.1 & 3.2.2).

There is still much to learn about the neurobehavioral toxicity of PBDEs in mammalian wildlife. In a recent review, Costa & Giordano (2007) concluded that subtle but lasting developmental neuroendocrine effects will occur at levels of PBDEs only marginally higher than currently found in animal tissues. Some of these effects are likely due to anti-thyroidogenic or brain cholinergic mechanisms. There are few if any studies examining this possibility. In a single ecological study on river otters, there were no correlations between cholinesterase activity and PBDE levels in the brain (Basu, Scheuhammer & O'Brien, 2007).

2.6.3.2 Non mammalian vertebrates

Methylmercury

Elevated exposure of fish and amphibians to methylmercury also impairs behaviours that are critical for successful reproduction, avoidance of predators and feeding (e.g. Weis, 2009). Laboratory studies have shown that, in general, the younger animals are more sensitive to the effects; for example, it takes 2-fold higher levels of methylmercury in adult than young fish to negatively affect behaviours (Beckvar, Dillon & Read, 2005). Similarly, in amphibians, maternal exposure negatively affected growth, duration of metamorphic climax, and swimming performance in a study of American toad

larvae. The duration of metamorphic climax is a period of increased vulnerability for immunological, energetic, and ecological reasons, and therefore mercury exposure at this time may increase mortality risk in exposed amphibian populations. It is interesting to note that the metamorphs from mercury-exposed mothers did not have elevated tissue concentrations due to dilution of maternally transferred mercury during growth.

PCBs

Over the last two decades Khan & Thomas (1997; 2001; & 2006) have accumulated substantial evidence on the involvement of PCBs in the disruption of the seratoninergic systems in fish brains. During gonadal recrudescence, PCBs reduce both dopamine and serotonin in various regions of the hypothalamus. This leads to an inhibition of the reproductive luteinizing hormone (LH) and impairment of gonadal growth. As in rats, this fall in dopamine and serotonin is thought to be caused by the inhibition of thyroid hormone induced by PCBs, and highlights the fact that adverse effects of endocrine disruptors on reproduction can also be due to their effects on neurohormones and thus indirectly on gonadal hormones.

PBDEs

In non-mammalian vertebrate wildlife, studies of neurodevelopmental disorders, occurring concomitantly with exposure to PBDEs, can be provided. There are multiple lines of evidence suggesting that PBDEs affect T_4 levels in developmentally exposed birds, fish and amphibians (e.g. Fernie et al., 2006; Lema et al., 2006; 2008; 2009) making it likely that these chemicals also affect neurodevelopment in these animals as in rodent models. The most dramatic effect reported in fish species is a hatching delay (Timme-Laragy, Levin & Di Giulio, 2006), which could be attributed to a T_4 mediated mechanism. Other possible T_4 mediated effects include those on tail curvature direction, hypo activity and elimination of the fright response, with important consequences for predator recognition and avoidance. Increases in thyroid hormone and its receptor occur just before hatching in zebra fish and can be altered by exposure to thyroid hormone receptor antagonists (Liu & Chan 2002).

2.6.4 Neuroendocrine effects of exposure to endocrine disrupting chemicals on courtship behaviour and mate choice in wildlife

2.6.4.1 Methylmercury

Studies of aquatic birds suggest that methyl mercury exposure at environmentally relevant levels can interfere with reproductive success- due either to overt neurotoxicity or more subtle neuroendocrine disruptive effects on courtship behaviour and mate choice. For example, common loons exposed to methylmercury showed increased lethargy, reduced time incubating the nest and foraging and feeding their young (Evers et al., 2008). As a result, adults in areas with higher exposure had decreased hatching success of eggs and production of chicks (Scheuhammer et al., 2007; Evers et al., 2008). High mercury levels in eggs have been suggested as a cause of declining ivory gulls in the Canadian Arctic (Braune, Mallory & Gilchrist, 2006). Moreover, methylmercury-exposed white ibises in the field and in the laboratory have shown altered testosterone and estradiol levels (Frederick & Jayasena, 2011), as well as altered courtship behaviour, altered song (Hallinger et al., 2010), high levels of male-male pairing and reduced reproductive success in successful pairs that did raise young (Frederick & Jayasena, 2011). Male to male pairing has been reported extensively in many animal species but it is most commonly associated with skewed sex ratios or limited mating opportunities. It is notable that this recent study did not report either of these conditions; mating opportunities and sex ratios were approximately equal. Moreover, male to male pairings do not normally occur in wild ibises. The exposure levels encountered in Frederick and Jayasena's study were environmentally relevant and are therefore of relevance to many bird populations. As reproductive output was decreased by both homosexual behaviours and as a result of a reduced number of fledglings raised by heterosexual pairs, mercury exposure could lead to altered demographic patterns in wild bird populations (Burgess & Meyer, 2008; Barr, 1986). Indeed, in the Frederick study, the breeding population size was inversely correlated with their annual methylmercury exposure in South Florida, USA (Frederick & Jayasena, 2011).

2.6.4.2 DDT

DDT is a persistent, widespread environmental contaminant found in most regions of the world and at high concentrations in countries where it is still used to control malaria mosquitoes (Chapter 3.2.1). The most well documented effects of DDT on neurobehaviour are those seen in birds, where DDT has been associated with decreased courtship behaviours (Zala & Penn, 2004), altered singing (in songbirds) and female to female pairing (in gulls). It is well known that administration of testosterone or estradiol to adult female or male birds, respectively, leads to mate attraction and courtship behaviour typical of the opposite sexes. In addition, early developmental exposures to estrogens or aromatase inhibitors has been shown to profoundly increase male and female sexual interest in the same sex and decrease male vigour. Sexual dimorphism of singing is also thought to be controlled not only by sexually dimorphic genes expressed in the brain but also by estrogen (reviewed in Adkins-Reagan, 2011); reducing the concentration of either 17β-estradiol or testosterone reaching the song system in the brain (a set of interconnected brain regions that mediate the learning, perception and vocalization of song) reduces the size of this area, concomitant with decreases in singing activity and song repertoire (Gulledge & Deviche, 1997; Metzdorf, Gahr & Fusani, 1999; Riters et al., 2000, Riters & Teague, 2003;). It is reasonable to hypothesise then that the effects of DDT on neurobehaviour in birds are at least partially due to its ability

to bind to and activate or inactivate the estrogen and androgen receptors, respectively, that are present at high concentrations in the song centre and two other areas of the brain (the ICo and the septum; Gahr et al., 2001). Both the size of the ICo and its neurons, thought to play a role in copulation, vocal displays and antagonistic behaviour, can also be affected by circulating hormone levels (Gurney & Konishi, 1980). Indeed, in a recent study, exposure to DDT (15-175 µg/g) during the embryonic and early post hatching period was shown to alter the structure of the American robin brain, such that relative forebrain size (male) and absolute song nuclei (male) and ICo volumes (male and female) were significantly reduced with increasing DDT exposure when the animals were examined 2 years post exposure (Iwaniuk et al., 2006). Although stress and direct neurotoxicity could not be ruled out as causes of these changes, it seems likely that endocrine disruption played a role, as the size of other adjacent AR and ER negative areas of the brain were not affected by the exposure.

Female-female pairing, as occurred when gulls, albatrosses and geese were exposed to DDT and other pesticides, could also have been due to feminization of the brain. In this case, however, these seemingly altered preferences could also have been opportunistic responses to a lack of availability of males (seen commonly in other species) and here caused by biased sex ratios in the gulls (also an effect of the DDT; see section 2.3 of this chapter).

2.6.4.3 Other EDCs

Feminizing effects of other xenoestrogens on sexually selected neurobehavioral traits have also been observed in wild mammals. A study of polygamous deer mice developmentally exposed to the endocrine disrupting chemicals ethinylestradiol (EE2) or bisphenol A (BPA) showed that although there were no changes in external phenotype, sensory development, or adult circulating concentrations of testosterone or corticosterone, spatial learning abilities and exploratory behaviours were compromised. Moreover, both BPA-exposed and control females preferred the control males in preference to the exposed males (Jasarevic et al., 2011). Males of this species compete for mates by expanding their territorial range during the breeding season, thereby increasing their prospects of locating mates that are widely dispersed. This adult male spatial ability and exploratory behaviour requires both a seasonal increase in testosterone and prenatal exposure to the same hormone (Galea, Karaliers & Ossenkopp, 1996).

The mechanism underlying the effects of ethinylestradiol (EE2) and BPA is not known, but could be either a direct effect of these EDCs on brain development in the male pups, and/or due to a decreased maternal investment in the pups by the dam (as has been observed also in rodents; Palanza et al., 2002; Della Seta et al., 2008). These changes could also occur as a result of effects on the expression of estrogen receptor genes during neurodevelopment or as a result of suppression of fetal testosterone production at the time when the androgens from the testes normally masculinize the developing brain.

The results of this study in a wild mammal are supported by numerous studies in rodents in the laboratory in which estrogenic chemicals have been shown to influence neurobiology. For example, several studies indicate that exposure of the developing brain to phytoestrogens affects early sexual differentiation of the brain, by mimicking effects of estrogens on the size and neurochemistry of sexually dimorphic regions causing alterations in reproductive behaviour. These effects are sensitive to the timing and duration of the exposure and inconsistent results are often noted due to differences in methodology. Moreover, increasing evidence also suggests PBDE exposure can also influence reproductive behaviours in birds at environmentally relevant concentrations (Fernie et al., 2008). It has been suggested that these studies are of relevance to human babies consuming soy formula during the early postnatal period (reviewed in Dickerson & Gore, 2007).

PCBs do not appear to influence adult volume of the sexually dimorphic areas of the brain but they can influence numbers of nuclear hormone receptors in these sexually dimorphic brain regions of exposed rodents during early development. It is not clear what these changes mean in terms of the function of the brain in this case. In addition, it is not yet clear whether the PCB congeners have equivalent potential for disruption of the sexual differentiation of the brain. Despite this, laboratory studies have consistently shown that PCB exposure during early development affects adult female reproductive behaviour and a limited number of studies also report effects on the male (reviewed in Dickerson & Gore, 2007).

Increasing evidence now indicates that xenoestrogens can affect sexualization of the brain in fish in a general manner, and not only sexually dimorphic features. Further study of this area is needed both in wild populations of fish and in biomedical toxicology where the fish brain could be considered a good model for brain sexualisation in humans. Unlike in mammals and birds, the brain of fish is not permanently sexualised during early development, but is instead, highly susceptible to hormones throughout its life. Despite this unique difference between fish and other vertebrates, most of the hormones sustaining the neurobehavioral controls of the reproductive process are similar, if not identical. Moreover, as recently discovered in mammals and birds estrogen, and not androgen, receptors in male and female fish play a role in differentiation of the neural circuits that control male-specific behaviour. The masculinizing effect of testosterone on the brain is through its conversion to estrogen by brain aromatase, a highly sensitive target for endocrine disruptors (reviewed in Le Page et al., 2010).

2.6.5 Evidence for a common EDC mechanism of neurodevelopmental disruption for humans and wildlife

In many cases, the neurotoxicological outcomes of chemical exposures are similar in wildlife and in humans, adding weight of evidence to relationships between chemical exposure and neurodevelopmental disorders in humans (Basu & Head, 2010).

Unlike in humans, pollutant levels in the brains of wildlife species can be easily sampled and measured and can reach levels that are associated with neurotoxic damage in humans. There are reports in the literature of damaged brains and spinal cords in wild bald eagles and great blue herons in the USA and a strong association between these anomalies and exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which was discovered at high levels in the birds. As early as 1915, lead-related neurological disorders were observed in horses and cattle living near industrial facilities. Moreover, before adverse neurological effects of mercury were seen in the human residents of the Great Lakes basin and in Minimata Bay (Japan), neurological effects were seen in local wildlife species through the 1950s and 1960s (Harada, 1995).

Wildlife can also provide important insights into mechanisms of neurotoxicity that may be important for human health. As one might expect from the high conservation of brain pituitary functions in vertebrates, neurohormones and neuropeptides controlling these tropic functions are well conserved and so the control of brain development by thyroid hormones and of reproductive behaviour by sex steroid hormones and GnRH neurons and neurotransmitters is similar amongst all vertebrates. Most of this work has been carried out in birds and fish, in which a diverse array of hormones have been shown to be involved in the stimulation of courtship and mating behaviour, including gonadal sex steroids produced locally within the brain, and neuropeptides.

2.6.6 Main messages

- Neurobehavioural disorders have increased in prevalence in human populations. The reasons for this are multiple and not understood.
- Despite this, the economic, societal and personal costs of this particular disease burden are high.
- There are some very strong datasets, for PCBs, showing that environmentally relevant exposures to these endocrine disrupting chemicals caused cognitive and behavioural deficits in humans.
- Studies of exposed wildlife provide important information on exposure levels, early and sub-clinical effects and clinical neurotoxicity of endocrine disrupting chemicals because the mechanisms underlying effects and the outcomes of exposures are often similar to those in humans. Wildlife data exist for some EDCs (e.g. PCBs) and potential EDCs (e.g. Mercury), but for other EDCs they are sparse or non-existent.
- Limited evidence shows that environmentally relevant exposures to some endocrine disrupting chemicals (mercury, bisphenol A, PCBs, PBDEs) could affect brain sexualisation, courtship behaviour and mate choice in some wildlife species, possibly leading to impacts at the population level.
- Chemical testing strategies do not routinely require evaluation of the ability of a chemical to produce developmental neurotoxic effects in a pre-market setting.
- New criteria for evidence are needed so that the scientific community and government agencies can focus their work and their funding on providing the most effective datasets required for regulation.

2.6.7 Scientific progress since 2002

Since the IPCS (2002) review on endocrine disruptors, the following advances have been made:

- Increased evidence for thyroid hormone mechanisms in brain disorders in humans and wildlife.
- Increased evidence of the great sensitivity of embryonic and postnatal development to EDCs when compared with adults.
- Increased number of studies showing a relationship between cognitive function and chemical exposures in humans. These studies however are often weakened by the nature of the study designs, and more prospective studies are warranted.
- Increased evidence for wildlife exposures to methylmercury and of effects on growth and development.
- First evidence of subtle effects of methylmercury and bisphenol A on reproductive behaviours of wildlife individuals that may be of relevance to populations.

2.6.8 Strength of evidence

There is sufficient evidence to conclude that published estimates of incidence and prevalence of some childhood neurobehavioural disorders have increased world wide over the past 10-20 years. Moreover, there is sufficient evidence to conclude that a number of factors, including environmental, contribute to the increases in autism spectrum disorders. There is also sufficient evidence to conclude that exposure to some industrial chemicals is plausibly related to the production of neurobehavioural disorders seen in both wildlife and humans. Exposures to lead, methylmercury, and PCBs represent strong cases in support of this, among others. There is sufficient evidence to conclude that PCBs can exert developmental neurotoxic effects in animals at doses that are similar to those of humans. More recent rodent studies of very low exposures to individual PCB congeners clearly make this point. There is sufficient evidence to conclude that specific PCB congeners and their metabolites can directly interfere with biological systems in rodents including thyroid hormone action and calcium regulation. There are limited data supporting an endocrine mechanism in the association of neurobehavioural disorders with some industrial chemicals. This is a challenging area that needs further focus. There are limited data to show that developmental exposure of some wildlife species to environmentally relevant concentrations of some chemicals can cause effects on brain sexualisation, leading to alterations in mate choice and courtship behaviours with outcomes that are relevant to populations. This area is important and needs further study.

2.6.9 References

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2.7 Endocrine disruptors and hormone-related cancers

2.7.1 Overview of hormon related cancer trends in humans and wildlife and evidence for endocrine disruption

This section deals with cancers of hormone-sensitive tissues such as the breast, uterus, ovaries, prostate and thyroid and considers the strength of the evidence for a contribution of endocrine disruptors to these diseases. Testis cancer is discussed in the section devoted to male reproductive health.

The role of steroidal hormones in various cancers has been a topic of intensive research from the early 1940s onwards. Although this work has established the biological plausibility of a strong involvement of endogenous estrogens and androgens in the disease processes, the possible contribution of foreign chemicals has only fairly recently received attention for two main reasons:

- Hormonal cancers of the breast, endometrium, ovary, testis prostate, and thyroid glands are continuing to rise among populations of "Western countries", and more recently also among Asian nations. Established risk factors alone cannot provide explanations for these unfavourable disease trends.
- The involvement of the synthetic estrogen, DES, in vaginal cancers and breast cancer has heightened concerns that a multitude of other hormonally active chemicals in everyday use are causing these diseases.

By far the most research into associations with endocrine disruptors has been carried out with breast, prostate and testis cancer, while other hormone-related cancers such as endometrial, ovarian and thyroid cancer have received very little attention.

Hormonal involvement in endocrine cancers

Despite a great deal of research, the etiology of most hormone-related cancers remains a mystery. It seems clear that hormones are necessary for the growth of cancerous tissues, but their involvement in the earlier steps of carcinogenesis is still unclear. The dominant theories of carcinogenesis invoke mutations as the ultimate cause of cancer, but most hormones are not strong mutagens (Soto & Sonnenschein, 2010). More recently, the field of epigenetics (Chapter 1.3.6) has begun to throw new light onto the processes that might contribute to hormonal cancers. It appears that mis-timed exposure of tissues to hormonally active agents can interfere with the subtle processes of gene-silencing, and that disruption of these processes might be one factor that predisposes towards cancer (see the review by Zhang & Ho, 2011). Other factors may include the disruption of tissue organization and differentiation during development (Soto & Sonnenschein, 2010).

2.7.2 Evidence for endocrine disruptor causation of hormonal cancers in humans and in rodent models

2.7.2.1 Breast cancer

Breast cancer incidence rates are increasing in almost all industrialized countries (WHO, 2010; Hery et al., 2008; **Figure 2.15**). These trends are not fully explained by improvements in diagnosis through mammographic screening (Coleman, 2000), nor in terms of changes in established risk factors, such as age at menarche or menopause, inherited susceptibility or increasing age of having babies. Twin studies have highlighted the importance of environmental factors, including chemical exposures (Lichtenstein et al., 2000; Luke et al., 2005).

Hormonal mechanisms of breast cancer

The cyclical secretion of estrogens during a woman's life is a key risk factor for breast cancer; the more estrogen one receives during life, the higher the overall risk (reviewed by Travis & Key, 2003). Neoplasms of the breast sequester estrogens which they require for their growth and hence, in order to demonstrate a link between breast cancer and exposure to estrogens, samples from women must be collected before they develop breast cancer. The lack of wide appreciation of this fact has led to many poorly designed and conflicting studies; the link between estrogen exposure and breast cancer was finally corroborated through nine prospective case control studies (The Endogenous Hormones and Breast Cancer Collaborative Group, 2002).

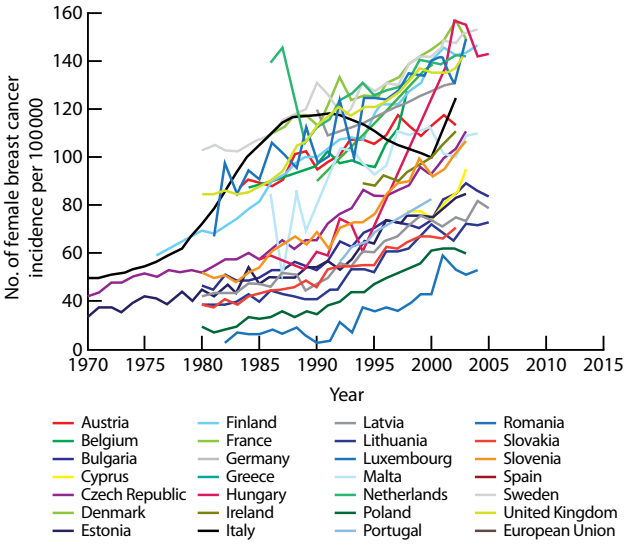

Figure 2.15. The rise in the number of new breast cancer age-standardized cases in several countries. All data from World Health Organisation (WHO), 20010, European health for all database (HFA-DB), World Health Organisation Regional Office for Europe. Database online at http://data.euro.who.int/hfadb/

The majority of breast cancers derive from the end buds of the breast, where the cells that contain estrogen receptors are the most responsive to estrogens (Russo & Russo, 2006). Ovarian estrogens signalling through estrogen receptors are also essential for the development of the breast during puberty, stimulating cell division in the "end buds" of the breast ducts leading to more "tree-like" branching and elongation of the ducts with every menstrual cycle. Although the exact mechanisms through which breast cancer is initiated by estrogens are still unclear today, one theory proposes that breast cancer cell populations arise from the well-established estrogen receptor-mediated proliferation of small numbers of incompletely differentiated cells in the end buds of the breast (Russo & Russo, 1998; Travis & Key, 2003). Other possibilities include direct genotoxicity (Liehr, 2001), aneuploidy induction (Russo & Russo, 2006; Liehr, 2001) and abnormal tissue remodelling through interactions between the stromal and epithelial tissues of the breast (Soto & Sonnenschein, 2010).

Epidemiological evidence that EDCs cause breast cancer

The breast is particularly vulnerable to cancer-causing influences during development in the womb and during puberty (Soto et al., 2008). Women whose mothers used the drug DES during pregnancy to avoid the risk of miscarriages (see section 2.1) have a high breast cancer risk (Palmer et al., 2006). Studies with laboratory animals also suggest that exposure to xenoestrogens during development can alter the development of the mammary tissue with possible consequences for breast cancer (Munoz-de-Toro et al., 2005; Maffini et al., 2006; Murray et al., 2007).

Natural and therapeutically used estrogens strongly contribute to breast cancer risks (Travis & Key, 2003). In particular, a meta-analysis of a large number of hormone replacement therapeutics (HRT) studies and trials done world wide concluded that estrogen-only HRT is associated with breast cancer (Grieser, Grieser & Doren, 2005). Moreover, the UK Million Women Study also showed that all forms of HRT, including estrogen only and estrogen-progesterone types increased breast cancer risk contributing to an extra 20 000 breast cancer cases in the preceding decade alone (Banks et al., 2003). A more recent USA study also corroborates the claims made by the Million Women Study for estrogen-progesterone combined HRT (Li et al., 2008). As a result of the publicity surrounding these results, there has been a steep decline in HRT use and concomitant steep declines in estrogen receptor positive breast tumours in European and US populations of women above the age of 50 (Verkooijen et al, 2009; Robbins & Clarke, 2007; Gompel & Plu-Bureau, 2010). Although this decline may be partially attributed to enhanced screening procedures for breast cancer, the collective evidence points very strongly towards HRT being one of multiple risk factors for breast cancer.

The findings related to HRT have fuelled concerns about chemical exposures, especially to estrogenic agents, and their role in breast cancer. However, most published human studies addressing the issue of estrogenic chemicals suffer from weaknesses that complicate their interpretation. Often, exposures were not measured during the periods of heightened vulnerability (during development in the womb or during puberty), and the effects of simultaneous exposures to multiple chemicals were not considered; there is now good experimental evidence that estrogenic chemicals with diverse features can act together to produce substantial combination effects (Kortenkamp, 2007). It is therefore not surprising that studies where single estrogenic pollutants (e.g. DDE, DDT or various estrogenic pesticides) were considered in isolation have failed to demonstrate significant breast cancer risks (reviewed by Snedeker, 2001; Mendez & Arab, 2003; Lopez-Cervantes et al., 2004). The importance of combined exposures was highlighted in a Spanish study, in which breast cancer risks increased with rising total estrogen load in adipose tissue (Ibarluzea et al., 2004). This observation supports the idea that estrogenic environmental chemicals in combination contribute to breast cancer risks, just as do natural and therapeutically used estrogens.

The most convincing evidence for associations between environmental pollutants (some with endocrine disrupting properties) and breast cancer comes from several epidemiological studies involving agents devoid of estrogenic activity (PCDD/F, PCBs, organic solvents; reviewed by Brody et al., 2007). Moreover, where DDT/DDE exposures during earlier life stages (puberty) could be reconstructed, breast cancer risks became apparent (Cohn et al., 2007; see Chapter 3.2.2.6 for DDT measurements in mothers' milk). This echoes insights from the DES epidemiology where the importance of periods of heightened vulnerability during development became obvious (Palmer et al., 2006; section 2.1 in this document). There are indications that exposure to cadmium, an estrogen mimic, is associated with breast cancer (reviewed by Kortenkamp, 2011). Epidemiological studies of more polar xenoestrogens, such as UV filter substances and phenolic agents, are missing altogether. An association between in vitro exposure to bisphenol A (exposure to this chemicals is reviewed in Chapter 3 and a pattern of gene expression related with higher tumour aggressiveness suggests a role of more polar xenestrogens in tumour progression and poorer patient outcome (Dairkee et al., 2008). By adopting targeted research strategies which take account of the origin of breast cancer early in life (prospective design) and which consider exposures to a multitude of chemicals, these issues should be pursued further.

Evidence from animal studies that EDCs play a role in breast cancer

Many experimental systems exist for the study of breast cancer, but the development of a coherent framework for the interpretation of all of the available evidence is severely hampered by a lack of fundamental knowledge about the mechanisms involved in breast cancer, and the extent to which observations in experimental models are relevant to the human situation.

Many assays sensitive to estrogen receptor activation are available, but a direct link between receptor activation and breast cancer causation cannot be assumed so that the interpretation of a positive result in such assays is not clear.

The utility and value of the two year chronic carcinogenicity bioassay as a tool for the identification of human breast carcinogens has been questioned (Rudel et al., 2007). Rudel and colleagues identified 216 chemicals as mammary gland carcinogens based on the outcome of this animal bioassay. However, the rodent strains used for these assays, the F344/N rat and the B6C3F1/N mouse, were not developed as models for the demonstration of mammary carcinogenesis; the assay aims to identify the ability of test chemicals to induce tumours, regardless of specific tissues. This complicates the interpretation of assay outcomes: an animal mammary carcinogen may be a human carcinogen but not necessarily with the breast as the target organ.

In a rat strain not routinely used for carcinogenicity testing, the ACI rat, DES, estradiol and other steroidal estrogens were found to induce mammary tumours (Shull et al., 1997; Ravoori et al., 2007). Equine estrogens used in hormone replacement therapy are also active (Okamoto et al., 2010). Evidence for an estrogen receptor-mediated mode of action in this model stems from the observation that estradiol-induced mammary neoplasms could be suppressed completely by co-treatment with the estrogen receptor antagonist tamoxifen (Li et al., 2002).

The ACI rat seems to be valuable tool for the identification of mammary tumours induced by estrogenic agents, yet, to our knowledge, other chemicals with estrogenic activity have not been tested in these models.

Various research models have been developed to explore the developmental anomalies that increase the susceptibility to mammary gland neoplasia later in life (summarized by Soto & Sonnenschein, 2010). The xenoestrogen bisphenol A has been used as a tool to explore these processes. It appears that exposure to bisphenol A during organogenesis induces profound alterations in the mammary gland that render it more susceptible to neoplasia. The accelerated maturation of the adipose tissue pad may be responsible for the epithelial changes and make the epithelium more sensitive to estrogens at later developmental stages. Consequently, increased sensitivity of the mammary glands to estradiol at puberty was observed in these animals, followed later by intraductal hyperplasia (a precancerous lesion) and carcinoma in situ (Durando et al., 2007; Murray et al., 2007; Vandenberg et al., 2008). Similarly, exposure to bisphenol A during nursing, followed by a challenge with DMBA produced increased numbers of tumours per rat and a shortened latency period (Jenkins et al., 2009).

2.7.2.2 Endometrial cancer

Endometrial cancer is the sixth most common cancer in women worldwide and, in industrialized countries, endometrial cancer is one of the most common cancers afflicting the female reproductive tract. The lowest rates were observed in South-Central Asia and Africa (excluding South Africa). Endometrial cancer was eight times more common in North America than in parts of Africa (**Figure 2.16**). In many countries, incidence has been increasing steadily over the past years (Kellert et al., 2009; Lindeman et al., 2010; Evans et al., 2011) such that around 288 000 cases of endometrial cancer were recorded in 2008.

There are two types of endometrial cancer, an estrogen-dependent variety, and one not dependent on estrogen. The increases in incidence seem to be limited to the estrogen-dependent type (Evans et al., 2011).

Endometriosis is most frequently diagnosed in post-menopausal women. As seen with breast cancer, elevated levels of endogenous sex hormones including total and free estradiol, estrone, and total and free testosterone are associated with increased risk (Allen et al., 2008). Not surprisingly, pharmaceutical estrogens used in combination with progestagen

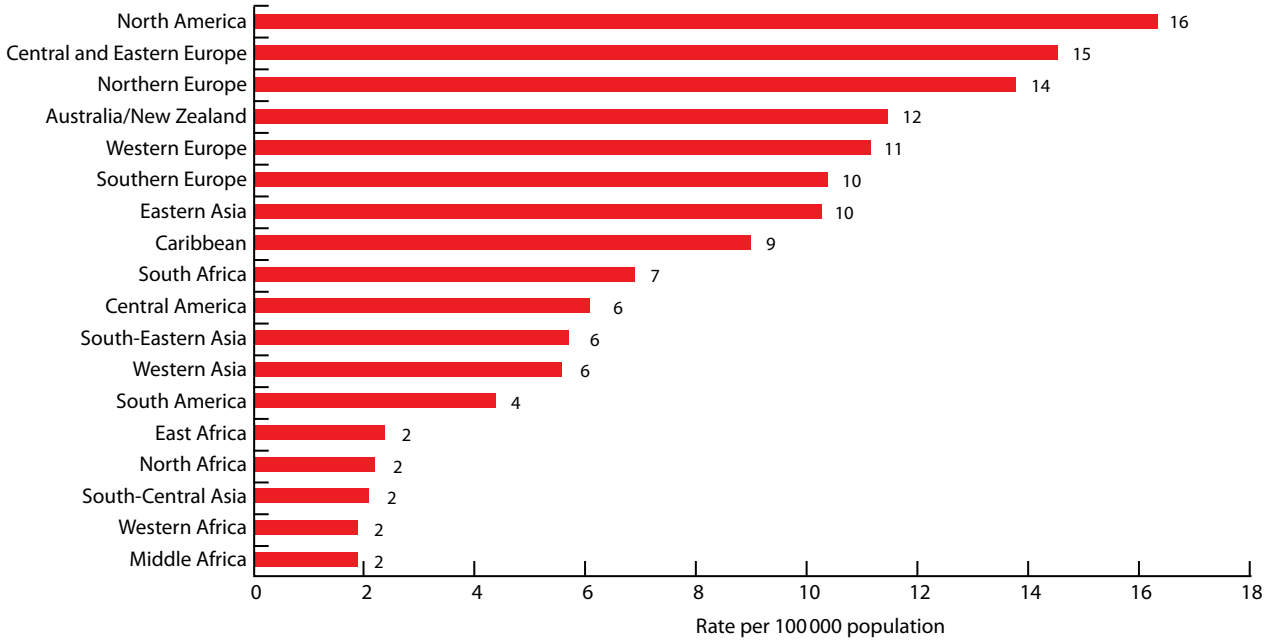

Figure 2.16. World wide age-standardized incidence rates for endometrial cancer in 2008 (Source: GLOBOCAN 2008, http://globocan.iarc.fr, Webpage:http://www.wcrf.org/cancer_facts/Endometrial_cancer_rates.php).

as hormone replacement therapy during menopause increase endometrial cancer risks (Jaakola et al., 2011).

Hormonal mechanisms of endometrial cancer

Most endometrial cancers are tumours that arise from tissues in the endometrium that peel off and regenerate repeatedly every month. Genetic alterations that cause endometrial cancers are, therefore, likely to arise in non-shedding cells, otherwise they would be lost with shedding during every menstruation cycle. It was recently hypothesized that these cells may be a type of stem cell (Kyo, Maida & Inoue, 2011).

The incidence of endometrial hyperplasia or adenocarcinoma is highly associated with prolonged unopposed estrogen action, often resulting from insufficient progesterone (Kim & Chapman-Davis, 2010). Indeed, a recent study found that the expression levels of over 100 genes known to be regulated by estrogen receptor α were also altered in the neoplastic uterus of mice, thus mimicking a hyper estrogenic environment. Emerging laboratory data also suggest that elevated levels of prostaglandin E_2 may underlie the transformation of normal endometrium to neoplastic tissue (e.g. Modugno et al., 2005). In both rodents and humans, deregulation of growth factor (IGFs) pathways and activation of phosphorylating enzymes are also characteristic of endometrial hyperplasia (McCampbell et al., 2006). Moreover, growth factors (e.g. IGF-II) are known to be targets of epigenetic gene silencing. Loss of imprinting of IGF-II resulting in its over expression, occurs in endometrial carcinosarcoma and may, therefore, contribute to abnormal endometrial proliferation characteristic of endometrial hyperplasia in both the rat and human. The complexity of mechanisms and risk factors for endometrial cancer are illustrated in **Figure 2.17**.

Epidemiological evidence that EDCs cause endometrial cancer

Although the involvement of estrogenic agents in the disease process of endometriosis would suggest risks also from estrogenic environmental chemicals, only very few investigations of that topic have been conducted. One of the earlier studies looked at possible associations with DDT serum levels, but produced inconclusive results (Sturgeon et al., 1998). In contrast, Hardell et al. (2004) found weak, but significant associations with serum DDE levels. Bisphenol A levels in patients with endometrial hyperplasia did not differ from those in healthy controls, but were lower in women suffering from hyperplasia with malignant potential (Hiroi et al., 2004). Some evidence also suggests that increased endometrial cancer risks could be linked to long-term cadmium intake (Akesson, Julin & Wolk, 2008).

Evidence from animal studies that EDCs cause endometrial cancer

Neonatal exposure of the developing rodent reproductive tract to xenoestrogens is a well-characterized model of hormone-dependent tumorigenesis in the uterus (Cook & Walker, 2004; Cook et al., 2005; Newbold, Bullock & Mclachlan, 1990; Walker, Hunter & Everitt, 2003) involving ERα-dependent mechanisms (Couse et al., 2001). Estrogen target genes induced in utero that persist into adulthood in DES exposed offspring, include c-fos and lactoferrin (Li et al., 1997; 2003). In the Eker rat model, neonatal DES exposure imparts a permanent estrogen imprint that alters reproductive tract morphology, increases susceptibility to develop uterine leiomyoma and induces endometrial hyperproliferative lesions in adult animals thought to be the precursors of endometrial cancer (Cook &

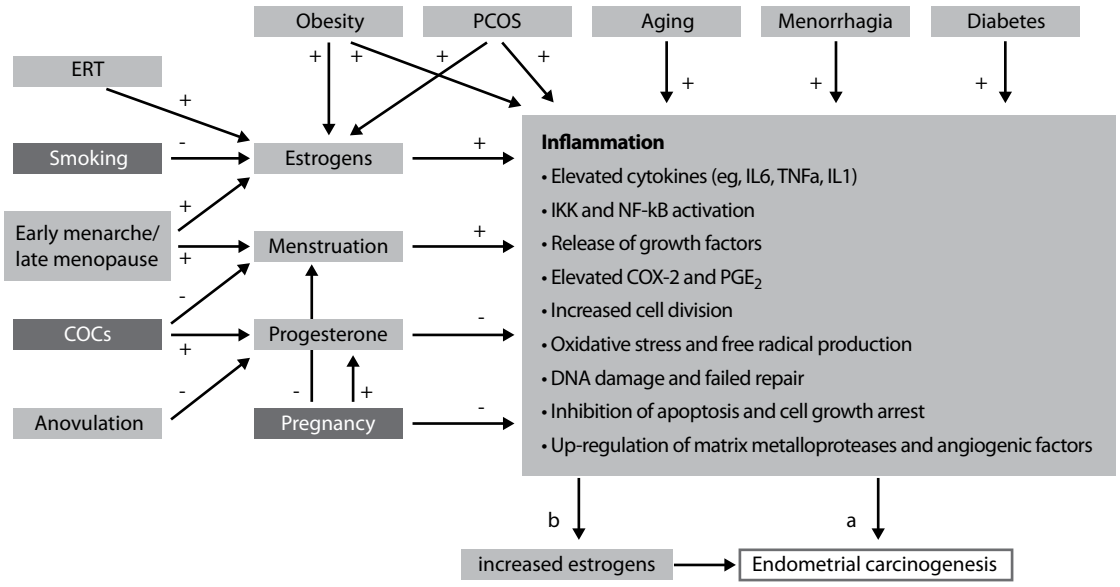

Figure 2.17. Proposed relationships among endometrial cancer risk/protective factors, inflammation, and endometrial carcinogenesis. Endometrial cancer risk factors either influence inflammation directly or influence factors that increase inflammation (e.g. estrogen, menstruation) or decrease inflammation (e.g. progesterone). Protective factors (in dark grey) exert the opposite effects. The effects of inflammation can cause mutagenesis, ultimately leading to endometrial carcinogenesis either directly (a) or indirectly (b) by increasing estrogen levels. ERT= unopposed estrogen therapy; COC=combined oral contraceptives; PCOS= polycystic ovary syndrome. (Figure from Modugno et al. (2005), redrawn; Used with publisher's permission)

Walker, 2004; Cook et al., 2005; 2007). Greater than 90% of CD-1 pups neonatally exposed to DES or the phytoestrogen genistein develop endometrial cancer by 18 months of age whilst C57BL/6 mice are resistant (Kabbarah, 2005). Few other xenoestrogens have been investigated for their ability to induce hyperplastic lesions of the endometrium. A recent study, however, showed that prenatal exposure of mice to bisphenol A elicited an endometriosis-like phenotype in the female offspring (Signorile et al., 2010).

2.7.2.3 Ovarian cancer

As with breast and endometrial cancer, the incidence trends for ovarian cancer are also pointing upwards (reviewed by Salehi et al., 2008). There are similarities with the risk factors important in breast cancer: increased age at menopause contributes to risks, while pregnancies are protective. Hormone replacement therapy increases the risks of developing ovarian cancer (Anderson et al., 2003; Beral et al., 2007).

The known role of estrogens in ovarian cancer indicates that endocrine disruptors might also unfavourably impact on risks, but very few studies of that issue have been conducted. An epidemiological association with exposure to triazine pesticides such as atrazine has been reported in one study (Young et al., 2005).

2.7.2.4 Prostate cancer

Prostate cancer is one of the most commonly diagnosed malignancies in European and USA men. Many countries, including all European countries, are experiencing dramatically increasing incidence trends, with the exception of high incidence countries such as The Netherlands and Austria (Karim-Kos et al., 2008; Jemal et al 2010; **Figure 2.18**).

Hormonal mechanisms of prostate cancer

Most prostate cancers derive from epithelial cells of the prostate gland, and androgens have long been established as playing a role in the causation of the disease (Huggins & Hodges, 2002). The involvement of estrogens has been recognised relatively late. Although estrogens, together with androgens, play a role in normal prostate development (Harkonen & Makela, 2004), estrogen exposure during fetal life can profoundly alter the developmental trajectory of the gland, sensitizing it to hyperplasia and cancer later in life (reviewed by Huang et al., 2004; Prins & Korach, 2008; Ellem & Risbridger, 2009).

Epidemiological evidence for EDCs causing prostate cancer

The upsurge in the incidence of prostate cancer in many countries has been attributed partly to changes in diagnostic methods, namely the introduction of prostate-specific antigen (PSA) screening, but this alone cannot explain the continuing rises. Changes in prostate cancer incidence among migrant populations and studies of twins show that environmental factors, including diet and chemical exposures, also contribute (Lichtenstein et al., 2000; Bostwick et al., 2004).

The spectrum of the environmental factors that may influence prostate cancer risks is, however, difficult to define; without a doubt dietary factors play an important role. In terms of chemical exposures, epidemiological studies have identified pesticide application in agriculture (Alavanja et al., 2003; Koutros et al., 2010), and pesticide manufacture (van Maele-Fabry et al., 2006) as issues of concern. Several current-use pesticides came to light as being associated with the disease,

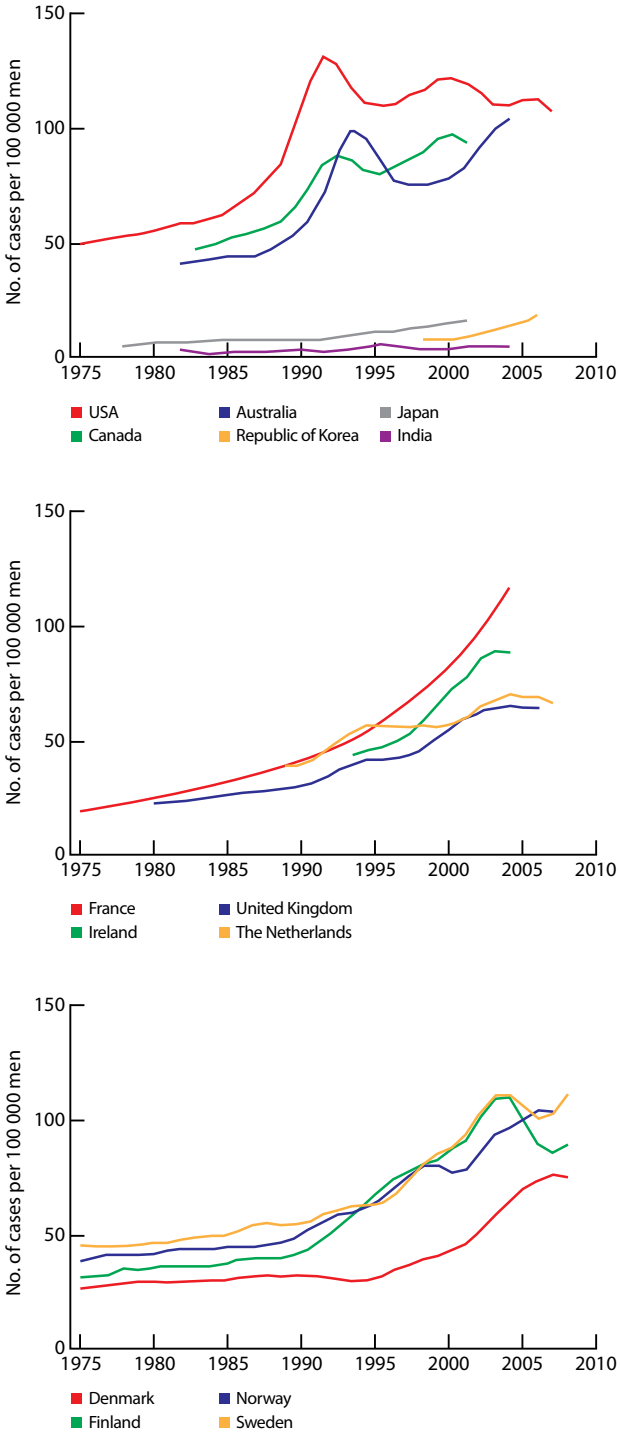

Figure 2.18. Trends in the incidence of prostate cancer in selected countries: age-standardized rate (W) per 100 000.
Source: http://globocan.iarc.fr/factsheets/cancers/prostate.asp

including methyl bromide, chlorpyrifos, fonofos, coumaphos, phorate, permethrin and butylate (see Chapter 3.1.1.6 for more information on these chemicals), the latter six only among applicators with a family history of the disease (Alavanja et al., 2003). Certain organochlorine pesticides, including oxychlordane (Ritchie et al., 2003), transchlordane (Hardell et al., 2006) and chlordecone (Multigner et al., 2010)(see Chapter 3.2.2 for review of human exposure to these POPs) were also found to be linked with increased prostate cancer risks.

Moderately chlorinated PCBs of the phenobarbital type, including CB -138, -153 and -180, could also be linked with prostate cancer, but there were no associations with PCB congeners of the co-planar, dioxin-like type (Ritchie et al., 2003; 2005). However, a Canadian case-control study among incident prostate cancer cases did not show any associations with PCB serum levels (Aronson et al., 2010).

Cadmium exposure (see Chapter 3.1.1.8 & 3.1.5.1 for a review of exposure and use) has been linked to prostate cancer in some, but not all epidemiological studies, and most positive studies indicate weak associations (Bostwick et al., 2004; Parent & Siemiatycki, 2001; Verougstraete, Lison & Hotz, 2003; Sahmoun et al., 2005). Arsenic exposure is strongly associated with prostate cancer (Benbrahim-Tallaa & Waalkes, 2008; Schuhmacher-Wolz et al., 2009).

The precise mechanisms by which the chemicals related to prostate cancer induce the carcinogenic process remain to be resolved. However, in the context of current understanding of the etiology of the disease, agents with androgenic, anti-androgenic and estrogenic activity are likely to be relevant. There is good evidence that the organochlorine pesticides shown to be associated with increased prostate cancer risks, including trans-chlordane, chlordecone, and trans-nonachlor, have estrogen-like activities (Soto et al., 1995). Cadmium also acts as an estrogen mimic, and arsenic seems capable of activating the estrogen receptor (Benbrahim-Tallaa & Waalkes, 2008).

Evidence from animal studies that EDCs cause prostate cancer

More than ten animal models for prostate carcinogenesis have been described (reviewed by Bostwick et al., 2004), but not one single model is able to re-capitulate the key features of the disease in men, which are 1) androgen dependence, 2) developing androgen-independence at more advanced stages, 3) slow growth, with long latency periods, and 4) able to metastasize to lymph nodes, bones and other organs.

In many rodent strains, including the F344 rat used for carcinogen testing, prostate tumours are not inducible by administration of androgens. Usually, tumours have to be "initiated" by exposure to genotoxic carcinogens such as nitrosoureas, followed by treatment with androgens in a "promotional" period.

The Noble rat is a good model for studying hormone-induced prostate cancers, but metastases are rare in this strain. This rat strain has not been widely used for the study of prostate cancers induced by chemicals.

Systematic screening exercises with endocrine disruptors for their ability to induce prostate cancers in animal models sensitive to hormonal prostate carcinogenesis have not been conducted, nor have international validation studies been initiated.

Many of the pesticides identified as being linked with prostate cancer are acetylcholine esterase inhibitors, and have not been shown to possess direct endocrine activity. However, they are capable of interfering with the metabolic conversion of steroid hormones and are thought to disturb the normal hormonal balance, with negative consequences for prostate cancer risks (Prins, 2008).

2.7.2.5 Testis cancer

Testis cancer is a relatively rare cancer, and the highest rates are reported in industrialized countries, particularly in western and northern Europe and Australia/New Zealand, (**Figure 2.19**). The incidence of testicular cancer is estimated to have doubled in the last 40 years, particularly in white Caucasians (also see **Figures 2.2, 2.3** and **2.4** of this Chapter 2.3). On average, the increases are 1-6% per annum and are reported for both seminomas and non-seminomas. The increasing trends appear to be influenced by birth cohort, with increasing risk for each generation of men born from the 1920s until the 1960s. For high risk countries there is evidence that the rate of increase has slowed over time and in several countries, including the UK, the most recent testicular cancer incidence rates have fallen slightly. Testicular cancer has an unusual age-distribution, occurring most commonly in young and middle-aged men, with its origin during fetal life. As such, it is discussed as part of the testicular dysgenesis syndrome in section 2.3.

2.7.2.6 Thyroid cancer

Although thyroid cancer is among the less common malignancies afflicting men and women, during the last few decades it has been increasing more rapidly than any other solid tumour. In most industrialized countries, the incidence of thyroid cancer has more than doubled since the early 1970s; for example 11 cases per 100 000 were diagnosed in the USA in 2006 (Sipos & Mazzaferri, 2008); Within the last two decades thyroid cancer has become the fastest rising neoplasm among women in North America (Holt, 2010). Similar trends have been observed in many other industrialized countries across the world (Cramer et al., 2010; Rego-Iraeta et al., 2009; Kilfoy et al., 2009; **Table 2.5**). Females, children and young adults are particularly vulnerable (Olaleye et al., 2011). Improvements in diagnostic histopathology are not regarded as the reason for the observed increases in thyroid cancer incidence (Cramer et al., 2010). There are several forms of thyroid cancer, defined in terms of their histology – follicular, papillary, and anaplastic. There is also medullary thyroid cancer. By far, anaplastic thyroid cancer is the most aggressive, with a mortality rate of nearly 100%.

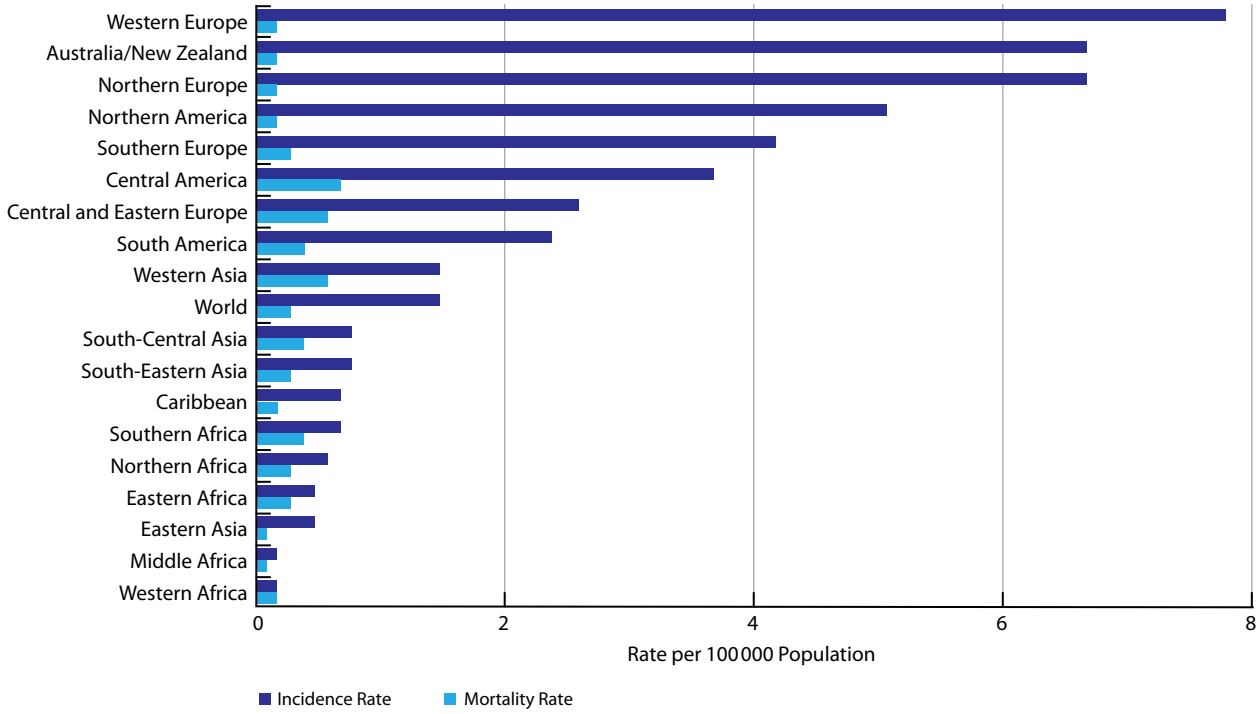

Figure 2.19. Testicular cancer, world age-standardized incidence and mortality rates, World Regions, 2008 (Figure from Ferlay et al. (2008), redrawn; Used with publisher's permission).

Table 2.5. International variation in thyroid cancer incidence rates, 1973-1977 to 1998-2002 (world age-standardized rates). (From: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2788231/table/T1/) Table printed with permission of the publisher.

	1973-1977				1998-2002				Males	Females
	Males		Females		Males		Females		%	%
	Cases	Rate	Cases	Rate	Cases	Rate	Cases	Rate	Change	Change
Europe, Scandinavian Countries										
Denmark	168	1	330	1.6	210	1.2	524	2.9	20.0	81.3
Norway	182	1.4	558	4.4	247	1.6	649	4.2	14.3	-5.8
Sweden	463	1.6	1158	3.9	407	1.3	1031	3.3	-18.8	-18.2
Finland	221	1.7	684	4.3	384	2.2	1281	7.0	29.4	62.8
Europe, Other										
France, Bas-Rhin	25	0.9	85	2.8	75	2.3	198	5.8	155.6	107.1
Switzerland, Geneva	18	1.9	43	3.5	27	2.0	98	6.5	5.3	85.7
UK, England, Thames	134	0.6	391	1.5	433	0.9	1133	2.3	50.0	53.3
Italy, Varese	45	2.0	105	3.8	45	2.9	123	7.1	45.0	86.8
Spain, Zaragosa	28	1.2	134	5.4	37	1.4	123	4.0	16.7	-25.9
Ocenia										
New Zealand	108	1.2	285	3.1	181	1.6	598	5.1	33.3	64.5
Australia, New South Wales	116	0.9	315	2.3	506	2.5	1639	8.1	177.8	252.2
Americas										
USA SEER[a]: White	997	2.3	2491	5.4	2216	3.5	6306	10.0	52.2	85.2
Canada, BC	104	1.5	252	3.6	271	2.1	733	5.6	40.0	55.6
Colombia, Cali	20	1.5	104	6.1	85	2.2	450	9.4	46.7	54.1
	(1972-1976)		(1972-1976)							
USA SEER[b]: Black	47	1.2	173	3.8	121	1.6	494	5.2	33.3	36.8
Asia										
China, Hong Kong	126	1.6	352	4.2	447	2.2	1557	7.2	37.5	71.4
	(1974-1977)		(1974-1977)							
Japan, Osaka Prefecture	129	0.7	432	2.1	432	1.3	1194	3.2	85.7	52.4
Singapore	43	1.3	141	3.8	180	2.0	636	6.6	53.8	73.7
Israel:Jews	193	2.6	472	6.2	474	3.5	1747	12.1	34.6	95.2
Africa										
Algeria, Setif					32	1.4	88	3.6		
Egypt, Gharbiah					53	1.1	151	2.6		
Tunisia, Center, Sousse					14	1.3	34	3.1		
Uganda, Kyandondo County					11	0.5	26	1.5		
Zimbabwe, Harare					14	1.0	45	3.1		

[a] Surveillance, Epidemiology and End Results

Mechanisms of thyroid cancer

According to a widely held view, thyroid cancer derives from well-differentiated normal cells (thyrocytes) by multiple changes in the genome (Giusti et al., 2010). This is supported by the fact that radiation is the biggest risk factor for this cancer and by the fact that there are large familial associations. However, clinical and molecular findings in thyroid carcinoma raise questions regarding the multi-step carcinogenesis hypothesis of thyroid cancer (Takano & Amino, 2005). There is little evidence to prove the succession of genomic changes, which casts doubt on the idea that aggressive carcinomas are derived from thyrocytes by accumulation of genetic changes. The alternative hypothesis proposes that thyroid cancer originates from the remnants of fetal, poorly differentiated, thyroid cells, and not from thyrocytes (Reya et al., 2001; Takano & Amino, 2005). Fetal thyroid cells have the ability to move through other cells, which is similar to the ability to induce invasion or metastasis. The existence of stem cells in the thyroid gland had been discussed often but identification in humans has not been successful. This is a knowledge gap.

In rodents, there is a strong influence of thyroid stimulating hormone on thyroid cancers. Persistent output of thyroid stimulating hormone (TSH) by the pituitary forces the follicle cells of the thyroid to divide in an effort to keep up with the demand for thyroid hormone. This then leads to hyperplasia and increased risk of cancer. In humans, however, it is not clear whether persistent TSH is a cause or a consequence of thyroid cancer.

In women of childbearing age thyroid cancer incidence is about 3 times higher than in men of similar age, and this suggests the possible involvement of estrogens and estrogenic chemicals (McTiernan, Weiss & Daling, 1984). There are however contradictory findings regarding the presence of estrogen receptors (ER) in thyroid cancers. While Kavanagh et al., (2010) have found ERα and β in thyroid tumours, the presence of ERα is disputed and the significance of the β form for malignant growth is unclear (Vaiman et al., 2010). Nevertheless, the higher incidence of thyroid cancer in women is attributed to the presence of a functional ER that participates in cellular processes contributing to enhanced mitogenic, migratory, and invasive properties of thyroid cells. In *in vitro* studies estradiol caused a 50-150% enhancement of the proliferation of thyroid cells (Rajoria et al., 2010). In rodents both TSH and estrogens stimulated thyrocyte proliferation (Banu, Govindarajulu & Aruldhas, 2002).

Epidemiological evidence that EDCs cause thyroid cancer

Although there is abundant evidence that several key components of thyroid hormone homeostasis are susceptible to the action of endocrine disruptors (see section 2.5), it is not clear whether chemicals that affect thyroid cell growth can lead to human thyroid cancer. Epidemiological studies investigating exposure to EDCs and the occurrence of thyroid cancer are scarce, and little work has focused on the possibility that environmental chemicals may contribute to some of the increased incidence of thyroid cancer (reviewed by Leux & Guenel, 2010). Especially in children, ionizing radiation is recognized as a key risk factor in thyroid cancer.

There are indications that thyroid cancer is associated with occupational exposure to solvents, with excess risks among females employed in shoemaking (Lope et al., 2005; Wingren et al., 1995). Women who worked as a dentist/dental assistants, teachers, or warehouse workers also were at risk (Wingren et al., 1995). Benzene and formaldehyde have been implicated as contributing to thyroid cancer risk (Wong et al., 2006).

An excess risk of thyroid cancer was observed in Swiss agricultural workers exposed to pesticides (see Chapter 3.1.5 for a review of what humans are exposed to); however, these risks could also have stemmed from an iodine deficit in the agricultural regions studied (Bouchardy et al., 2002). In the American prospective cohort on 90 000 pesticide applicators and their wives (Agricultural Health Study, AHS), there was an increased incidence of thyroid cancers when compared to the general population (Blair et al., 2005). Pesticide applicators engaged in handling the herbicide alachlor showed a moderate, but statistically non-significant, increase in thyroid cancer risk (Lee et al., 2004).

Follow-up studies after the 1976 Seveso accident report a suggestive, almost significant increase for thyroid cancer associated with 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (Pesatori et al., 2003). Thyroid cancer risk was also increased in a large occupational cohort of pesticide sprayers with possible exposure to dioxin (Saracci et al., 1991).

Associations with other thyroid diseases

It was recently documented that individuals with autoimmune thyroid diseases (discussed in sections 2.5, 2.11) such as Graves' disease or Hashimoto thyroididtis tend to have a much higher risk of developing cancer of the thyroid gland (Shih et al., 2008). A total of 50% of the 474 patients evaluated in this study had thyroid cancer, many more than the 28% who went into the surgery with a thyroid cancer diagnosis. The prevalence of thyroid cancer in the Hashimoto patients was 35.6%, and twice that found in the patients with Graves' disease. Likewise, participants with Hashimoto's thyroiditis were more likely to have benign thyroid adenomas, with advanced age being an especially strong risk factor. It is unclear why there is a link between Hashimoto thyroiditis and cancer, but it warrants further study.

Evidence from animal studies that EDCs cause thyroid cancer

A number of pesticides have been shown to induce thyroid follicular cell tumours in rodents, which according to the US Environmental Protection Agency (EPA) are relevant for the assessment of carcinogenicity in humans. Of 240 pesticides screened, at least 24 (10%) produced thyroid follicular cell tumours in rodents. Of the studied chemicals, only bromacil lacked antithyroid activity. Intrathyroidal and extrathyroidal sites of action were found for amitrole, ethylene thiourea,

and mancozeb, which are thyroid peroxidase inhibitors; and acetochlor, clofentezine, fenbuconazole, fipronil, pendimethalin, pentachloronitrobenzene, prodiamine, pyrimethanil, and thiazopyr, which seemed to enhance the hepatic metabolism and excretion of thyroid hormone (Hurley, Hill & Whiting, 1998).

The current understanding of the etiology of thyroid cancer does not clearly link it to an endocrine mechanism. However, chemicals disrupting the hypothalamic pituitary thyroid (HPT) axis and xenestrogens seem to be of importance at least in the progression of the disease. Therefore, the precise mechanisms of cancer causing action of the chemicals demonstrated in epidemiological studies to be related to thyroid cancer remain to be resolved. There is plenty of evidence that EDCs interfere with thyroid homeostasis through numerous mechanisms of action. Many substances exert a direct and/or indirect effect on the thyroid gland by disrupting certain steps in the biosynthesis, secretion, and peripheral metabolism of thyroid hormones (Boas et al., 2006). However, it is not clearly established whether chemicals that affect thyroid cell growth lead to human thyroid cancer. Investigations that compare the susceptibility to disruptors associated with thyroid cancer between rodents and humans would be useful. A recent review by Mastorakos (2007) summarizes substances that have been found to act as EDCs via the HPT axis in different species. Ten of the listed chemicals have been shown to cause an increased risk of thyroid neoplasms and tumours in rodents and the possible mechanisms are explained.

Mechanisms of thyroid cancer are heterogeneous and include gene mutations as well as the potential for a resident population of stem cells to become tumorigenic, or invasion of brain marrow-derived stem cells. This later process has been speculated to be sensitive to estrogen action which may explain the gender difference.

Several rodent two-step carcinogenesis models have been developed (Kitahori et al., 1984; Son et al., 2000; Takagi et al., 2002). These rodent carcinogenesis models are useful, as little is known about the development of thyroid cancer in general. However, thyroid cancer development in humans seems to differ from that in rodents in that the latter are more sensitive to increased TSH levels. There are also differences in the normal physiological thyroid hormone processes between rats and humans. In rats, 40% of T3 is secreted directly from the thyroid, compared to 20% in humans and the structure of the deiodinase enzyme in rats is different from that of humans (Takser et al., 2005). In humans, circulating thyroid hormones are primarily bound to thyroxine-binding globulin (TBG), with smaller amounts bound to albumin and transthyretin. In developing rats, TBG is not present in the circulation between months two and seven and adult rat thyroid hormones are primarily bound to transthyretin, and, to a lesser extent, albumin. These proteins have a lower affinity for thyroid hormones than TBG resulting in a shorter half-life of thyroid hormones in adult rats (Lans et al., 1994).

2.7.3 Evidence for endocrine disruptor causation of hormonal cancers in wildlife

Until relatively recently, cancer in wildlife species was not of particular concern, because it appeared to occur at lower rates in most wildlife species than in humans. However, with increased monitoring of particularly endangered wildlife species, the identification of Tasmanian devil facial tumour disease (a neuroendocrine cancer), sea turtle fibropapillomatosis and sea lion genital carcinoma, it has become apparent that neoplasias (including endocrine neoplasias) can be highly prevalent in wildlife and have considerable effects on populations of some species (McAloose & Newton, 2009).

2.7.3.1 Vertebrate wildlife

Little has been written specifically about hormonal cancers in animal species other than humans. The available literature suggests that most of the endocrine cancers of humans are known to occur as similar entities in dogs, cats and wildlife species. The rates of endocrine cancers in both domestic and wild animal species are generally lower than those observed in humans, but there are reports of increased rates of these and other tumours in some populations. In a review of cetacean tumours, for example, the reproductive tract was cited as one of the more common organ systems to be affected by neoplasia. If the reproductive tract is affected, this can interfere with successful breeding and parturition. In one study, benign genital papillomas were present in 66.7% of dusky dolphins and 48.5% of Burmeister's porpoises, a rate considered to be high enough to interfere with copulation in some cases (Van Bressem et al., 1996; Van Bressem, Van Waerebeek & Raga, 1999). Genital tract carcinomas were also reported to be increasing in California sea lions between 1979 and 1994, only rarely reported in any type of seal/sea lion prior to 1980 (Gulland, Lowenstine & Spraker, 2001; Sweeney & Gilmartin, 1974; Newman & Smith 2006); 18% of sexually mature sea lions found stranded on the California coast in 1994 had aggressive genital carcinomas (Gulland et al., 1996), a rate that is unprecedented in any pinnepid species.

Associations between exposure to anthropogenic contaminants and the development of neoplasia (but not particular endocrine neoplasias) in wildlife populations is difficult to study, but in certain monitored populations such as beluga whales of the St Lawrence estuary in Canada (reviewed in McAloose & Newton, 2009) and bottom-dwelling fish of the same region (Malins et al., 1985a; 1985b; Smith, Ferguson & Hayes, 1989; Bauman, Smith & Metcalf, 1996), cancers are quite well documented. In the St. Lawrence estuary beluga whales, monitored for a period of 17 years, the estimated annual rate of all cancers, including hormonal cancers (163/100 000 animals) is much higher than reported for any other cetacean population and similar to that reported in humans and hospitalized cats and cattle (Martineau et al., 2002). Endocrine

cancers including leiomyomas of the vagina, cervix and uterus, mammary adenocarcinomas and adrenal and thyroid tumours were found (Mikaelian et al., 2000). For all types of cancer, the annual incidence rate was higher than or equal to that found in any other animal population and highest for intestinal cancer. There is no evidence that cancer is normally frequent in beluga from less polluted environments, or that it is caused by old age (Martineu et al., 2002). Carcinogenic PAHs from aluminium smelters are, however, present in the environment of the St. Lawrence beluga and are likely ingested by these animals when feeding on benthic species of invertebrates. Systematic studies to assess the direct or potential roles of these contaminants have not been done, although most of the published examples strongly suggest that carcinogenesis in the beluga whale is a result of the combined effects of multiple factors, including exposure to PAHs present in their local environment (DeGuise, Lagace & Beland, 1994; DeGuise et al., 1995; Mikaelian et al., 1999; Martineau et al., 1988; 1995; Muir 1996a; 1996b; Hobbs et al., 2003; Chapter 3.2.1 contains a review of chemicals exposures in wildlife generally).

There are several other instances where the interplay between chemical contaminants and other factors in causing cancer in wildlife is clearly visible. In many of these cases, viruses are known causal factors (King et al., 2002; Buckles et al., 2006), but in some instances there is also evidence of a co-causal role played by chemical contaminants. These include increased incidence of fibropapillomatosis in sea turtles living in polluted bodies of water (Herbst & Klein, 1995; Foley et al., 2005; Work et al., 2004), an 85% higher level of PCBs found in the blubber of sea lions with genital carcinoma compared to those without this disease (Ylitalo et al., 2005), and an increased rate of epizootics of the liver and skin cancer in fish living in industrial waterways (Malins et al., 1985a; 1985b; 1987; Black & Baumann, 1991; Blazer, 2006; Sakamoto & White, 2002; Williams et al., 1994). Indeed, epidemics of liver cancer have been found in 16 species of fish in 25 different polluted locations, both fresh and salt water. The same tumours have been found in bottom-feeding fish in industrialized and urbanized areas along Canada's Atlantic and Pacific coasts, whereas in Canada's less polluted waters cancer in fish is reported to be almost non-existent.

Experimental support for relationships between environmental pollutant exposure and cancer in fish and mammals also exists. For example, laboratory models have demonstrated that exposure to the PAH, benzo(a)pyrene, produces liver and/or skin tumours in fish, depending on the route of exposure (Hendricks et al., 1985; Black, 1984). In rodents, a relationship between intestinal cancer and PAHs is supported by observations in mice, whereby chronic ingestion of coal tar mixtures (containing benzo(a)pyrene) causes small intestinal carcinomas. Moreover, associations have been made in fish populations where environmental contamination decreased concomitant with decreases in the cancer rates (Bauman, Harshbarger & Hartman, 1990; Bauman & Harshbarger, 1995).

2.7.3.2 Invertebrate wildlife

Little information is available on endocrine neoplasias in invertebrate species and even less information links any incidence of invertebrate neoplasia with contaminant exposure. Nonetheless, a field survey carried out in three geographically distinct populations of soft-shell clams in eastern Maine, USA identified a high incidence of gonadal tumours (Gardner et al., 1991) and at all three locations, exposure to significant concentrations of the herbicide, Tordon 101 (picloram), 2,4-D and 2,4,5-T had occurred. Although 2,4-D and 2,4,5-T are not known to be potent carcinogens, TCDD, a by-product contaminant from the synthesis of 2,4,5-T is (Schmidt, 1992). Other types of neoplasias (in the respiratory system and hemolymph) are commonly found in bivalves; at least 22 species of estuarine bivalve molluscs show neoplasias (sometimes in >90% of individuals) on both coasts of North America, in Australia and in several countries in South America, Asia, and Europe (Wolowicz, Smolarz & Sokolowski, 2005). Incidence of these neoplasias tends to be highest in molluscs living in more polluted sediments (Wolowicz, Smolarz & Sokolowski, 2005).

2.7.4 Evidence for a common mechanism of hormonal cancers in human and wildlife

In many cases, hormonal cancers in vertebrate wildlife and domestic animal closely resemble the corresponding human carcinoma in terms of clinical behaviour, pattern of circulating hormone levels and expression of hormone receptors in primary tumours. For example, the role of steroidal hormones in wildlife cancers has been best described in domestic and zoo animals, where the use of the progestin, melengestrol acetate, as a contraceptive has been strongly associated with both ovarian and mammary carcinomas, as well as endometrial hyperplasias in domestic dogs and cats, tigers, lions and jaguars (McAloose, Munson & Naydan, 2007; Harrenstien et al., 1996). This contraceptive prevents the animals from breeding, resulting in their being exposed to recurrent estrogen peaks followed by high persistent levels of progesterone. As for women, estrogen (ER) and progesterone receptor (PR) expression varies in canine and feline mammary cancers. In general, ER expression is low, but PR expression persists in most cancers. Alterations in molecular controls of cell proliferation or survival in breast cancer, as seen in humans, have been identified in dog and cat mammary cancers, making them excellent models for human breast cancer.

There is no evidence available to show that endocrine disrupting environmental contaminants that are hypothesized to play a role in the causation of specific human endocrine cancers also play a role in wildlife and domestic animal endocrine cancers. Notwithstanding this, the links between animal and human health are long-standing. Viral and chemical-induced oncogenesis is a familiar concept in both human and wildlife studies and the study of animal viruses has led to new insights

into the molecular mechanisms of human cancers. Moreover, differences in mammary cancer prevalence between carnivores and herbivores and between captive and wild carnivores are striking and support the hypotheses that diet and reproductive history are major risk factors for these cancers. In wildlife, relationships between tumour development and environmental contamination are strongly suggested by scientific data in some contaminated regions of the world. Similarities of high cancer incidence and tumour type between species would support the conclusion of common risk factors in shared environments and show the value of wildlife as important environmental sentinels. In the St. Lawrence estuary, for example, the human population is also affected by higher rates of cancer than populations in other parts of Quebec and Canada, and some of these cancers have been epidemiologically related to exposure to PAHs, as seen in the beluga whale. In another example, a study of more than 8 000 dogs showed that canine bladder cancer was associated with their living in industrialized countries, mimicking the distribution of bladder cancer among their human owners (Hayes, Hoover & Tarone, 1981). Further investigations of the role of pesticides in canine bladder cancer showed that the risk of bladder cancer was significantly higher among dogs exposed to lawns or gardens treated with herbicides or insecticides, including peony herbicides, but not among dogs exposed to lawns or gardens treated with insecticides alone. Moreover, risk of bladder cancer was higher if the dogs were obese and lived near another source of pesticides and lower if the diet contained green leafy vegetables (Glickman et al., 1989; 2004). A final example from a survey of cancer mortality rates in the United States indicated that the mortality rates due to ovarian and other reproductive organ cancers in human females from Washington County, Maine, and from Indian River, Florida were significantly higher than the national average (Riggan et al., 1987), coinciding with geographic areas in which tumour-bearing clam populations were also located (Gardner et al., 1991; Hesselman, Blake & Peters, 1988). It is also of interest that DNA from mollusc tumours is able to transform mammalian cells into cancerous cells in vitro (Van Beneden, 1994).

Taken together these observations suggest that human and animal populations may be affected by specific types of cancer because they share the same habitat and are exposed to similar types of contaminants. Many of the molecular mechanisms governing cancer are evolutionarily conserved between wildlife and humans and so this is a mechanistically plausible hypothesis. Although none of these examples particularly highlight endocrine cancers, there has been very little if any study of this topic.

2.7.5 Main messages

- The incidences of all endocrine-related cancers (breast, endometrial, ovarian, prostate, testis and thyroid) in humans are rising in many countries, or are levelling off at a high plateau.
- The increase in incidence of endocrine-related cancers in humans cannot be completely explained by genetic factors; environmental factors, including chemical exposures, are involved, but very few of these factors have been pinpointed.
- For breast, endometrial and ovarian cancer, the role of endogenous and therapeutical estrogens is well documented; this makes it biologically plausible that xenoestrogens might also contribute to risks. However, the EDCs shown to be associated with breast cancer risks (PCDD, PCBs and solvents) do not have strong estrogenic potential. There are indications that endometrial cancer is linked to the xenoestrogens DDE and cadmium.
- For prostate cancer in humans, weak associations with exposures to pesticides (occupational), PCBs, cadmium and arsenic have come to light. There is good biological plausibility that androgens and estrogens are involved in the disease process.
- For human thyroid cancer, there are indications of weak associations with pesticides and TCDD.
- For most of the hormonal cancers in humans, valid animal models are not available. This makes the identification of hormonal carcinogens very difficult, and forces researchers to rely on human epidemiological studies. However, epidemiological studies cannot easily pinpoint specific chemicals, and can identify carcinogenic risks only after the disease has occurred.
- A general weakness of the environmental epidemiology of hormone-related cancers has been a lack of focus on holistic exposure scenarios. So far, epidemiology in this area has explored quite narrow hypotheses about a few priority pollutants, without taking account of combined exposures to a broader range of pollutants.
- Cancers of endocrine organs, particularly reproductive organs, are also found in wildlife species (several species of marine mammals and invertebrates) and tend to be more common in animals living in polluted regions than in more pristine environments.
- Wildlife populations and domestic pets may be affected by the same types of cancers as humans because they share the same habitat and are exposed to similar types of contaminants. Greater study of this wildlife and domestic pets as environmental sentinels for hormonal cancers in humans is needed.

2.7.6 Scientific progress since 2002

Significant advances in our knowledge of hormonal cancers have occurred since the 2002 IPCS *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002). These include:

- In breast cancer, the vulnerability of breast tissue to cancer-causing influences during fetal life and puberty has been recognized.

- Where exposures during these life stages could be re-constructed, as was the case with DES and DDT, associations with elevated breast cancer risk later in life could be demonstrated. Combined internal exposures to non-steroidal estrogens are also risk factors in breast cancer. Taken together, this evidence strengthens the biological plausibility that other estrogenic chemicals are also contributors to risks, but adequate studies to prove this point have not been conducted.
- Steroidal estrogens are also risk factors in endometrial and ovarian cancer, but the involvement of other estrogenic chemicals remains to be elucidated.
- In prostate cancer, the importance of exposures to estrogens as risk factors has received attention. There is evidence that cadmium, arsenic and non-coplanar PCBs contribute to prostate cancer risks, as do exposures to unspecified pesticides among pesticide applicators. Whether there are hormonal mechanisms at work remains to be clarified.

2.7.7 Strength of evidence

There is sufficient evidence that the incidence of most hormonal cancers has increased or remains at a high level, and that environmental exposures play a role in these unfavourable trends. However, the nature of these environmental factors is poorly defined in terms of contributing chemicals.

Several independent studies have shown associations between PCDD/F exposures and elevated breast cancer risks (reviewed by Brody et al., 2007; Dai & Oyana, 2008). There is therefore sufficient evidence linking breast cancer with dioxins and furans (see Chapter 3.2.2 for a review of what humans are exposed to).

There is also sufficient evidence for increased breast cancer risks among women with elevated PCB exposures and a Cyp polymorphism (Brody et al., 2007).

Sufficient evidence exists for a link between pesticide exposures during application (Alavanja et al., 2003; Koutros et al., 2010) and manufacture (van Maele-Fabry et al., 2006) and prostate cancer. However, the nature of the implicated agents remains to be pinpointed.

One epidemiological study (Ibarluzea et al., 2004) has demonstrated a link between internal estrogen burden from lipophilic chemicals and breast cancer. Single epidemiological studies have shown associations between DDT and endometrial cancer, and between triazine pesticides and ovarian cancer. Because these observations have thus far not been replicated by others, the evidence is limited.

There is thus far no evidence linking thyroid cancer with any endocrine disrupting chemicals.

In general, the study of endocrine disrupting environmental pollutants and hormonal cancers is characterized by epidemiological studies that have pursued very narrow hypotheses about the contributing chemical substances. In many cases, investigations were driven by the availability of chemical analytical techniques, rather than by biologically plausible ideas about etiological factors. If epidemiology is to make a larger contribution to this field of study, the effects of combined exposures will have to be considered.

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2.8 Endocrine disrupting chemicals and adrenal disorders in humans and wildlife

2.8.1 Overview of adrenal function and dysfunction in humans and wildlife and evidence of endocrine disruption

2.8.1.1 Adrenal dysfunction caused by exposure to EDCs

Disorders relating to hyper-secretion of the hormone cortisol from the adrenal glands are classically referred to as Cushing disease/syndrome. Normally, adrenocorticotropic hormone (ACTH) is released from the pituitary gland in the brain to stimulate the release of the stress hormone cortisol from the adrenals. In Cushing disease, either a tumour in the pituitary secretes ACTH or excess cortisol is produced by adrenal gland tumours, or by hyperplastic adrenal tissues. Along with a suite of metabolic disturbances, including high blood sugar levels (hyperglycemia), redistribution of body fat and an increased protein catabolism, gross enlargement of the adrenal cortex is frequently observed in this disease. The reversed situation resulting from adrenal hypotrophy and hypo-secretion of adrenal steroid hormones may result in Addison disease, a potentially fatal condition depending on the extent of tissue destruction in the adrenal cortex.

The involvement of EDCs in adrenal health problems came into focus when frequent cases of massive adrenocortical hyperplasia and a suite of pathological lesions characteristic of Cushing disease were identified in all three species of seals inhabiting the Baltic Sea (Bergman & Olsson, 1985). The syndrome, also involving lesions in the female reproductive tract, the intestines, skin and skeleton, was associated with high body burdens of PCBs and DDT compounds in Baltic wildlife at the time (see Chapter 3.1.4 & 3.2.1 for a review of exposure of wildlife to DDT and PCBs).

Persistent aryl methyl sulfone metabolites derived from PCBs and DDT were originally demonstrated in the blubber of Baltic grey seals (Jensen & Jansson, 1976). Moreover, these compounds are now known to be widely present in the tissues of other marine animals as well as in human milk (Bergman et al., 1994; Haraguchi et al., 1992; Letcher, Norström & Bergman, 1995; Newsome & Davis, 1996; Noren et al., 1996; Troisi et al., 2000; Weistrand & Noren, 1997; Chapter 3.2.2). Subsequent research on this new class of POPs revealed that the DDT metabolite methylsulfonyl-DDE ($MeSO_2$-DDE) was a highly potent and tissue-specific toxicant that induced degeneration and necrosis in the adrenal cortex of laboratory mice following a single dose (Lund, Bergman & Brandt, 1988; Jönsson et al., 1991; Lund & Lund, 1995; Lindhe et al., 2001). Reduced plasma corticosterone levels were subsequently recorded in suckling mice following administration of $MeSO_2$-DDE to the lactating dam (Jönsson, Lund & Brandt, 1993). Representing a completely different mechanism of action, methylsulfonyl metabolites formed from PCBs ($MeSO_2$-PCBs) were found to act as antagonists following binding to the human glucocorticoid receptor (GR) *in vitro* (Johansson, Nilsson & Lund, 1998).

The exceptional adrenocorticolytic potency of $MeSO_2$--DDE *in vivo* in mice and the antagonistic binding of PCB-metabolites to the human GR *in vitro* triggered interest in other environmental contaminants with a capacity to perturb hormone secretion in the adrenal cortex of humans and wildlife species. Since adrenal steroid hormone synthesis is regulated through the feed-back system of the hypothalamic pituitary adrenal (HPA) axis, it is highly responsive to various types of stress, including handling. It is therefore difficult to accurately measure plasma levels of the stress hormones cortisol and corticosterone in free-ranging wildlife as well as in experimental animals and relate them solely to exposure to chemical exposures. In current research efforts, all components of the HPA axis, rather than only the glucocorticoid-secreting adrenal cells, have come into focus.

Numerous chemicals affecting adrenal structure and function have been described (Hinson & Raven, 2006). Due to its high blood supply and lipid content, a variety of persistent environmental contaminants and other chemicals are selectively taken up and retained in the adrenal cortex, both in adults and fetuses (Brandt, 1977). Due to a high expression of cytochrome P450 (CYPs), the cortex also has a pronounced ability to metabolize specific chemicals to reactive and toxic intermediates that become covalently bound to cellular macromolecules and subsequently degenerate or kill the cells in which they were formed. Since adrenal CYPs are expressed also in fetuses and neonates, a high toxicity in the fetal and neonatal adrenal cortex may occur following trans-placental transport and exposure via milk (Jönsson, Rodriguez-Martinez & Brandt, 1995; Jönsson et al., 1992).

2.8.1.2 The adrenal gland and its hormones

The mammalian adrenal glands are small, pyramid-shaped organs, situated on top of the kidneys. They consist of an inner medulla and an outer cortex. The adrenal medulla secretes catecholamines like adrenalin and noradrenalin, while the three histologically and functionally distinct layers (zonae) of the adrenal cortex secrete a variety of steroid hormones (corticosteroids), many of which are essential for survival: the outer *zona glomerulosa* synthesizes mineralocorticoids, the large middle *zona fasciculata* synthesizes glucocorticoids, and the inner *zona reticularis*, which does not fully develop until puberty, produces androgens. The functional zonation of the adrenal cortex is a consequence of layer-specific expression of steroidogenic genes: the resulting enzymatic repertoire determines the fate of cholesterol in a given *zona*.

Steroidogenesis

Steroid hormones of the adrenal glands are synthesized from cholesterol via activation of a number of enzymes and regulatory proteins in both the mitochondria and

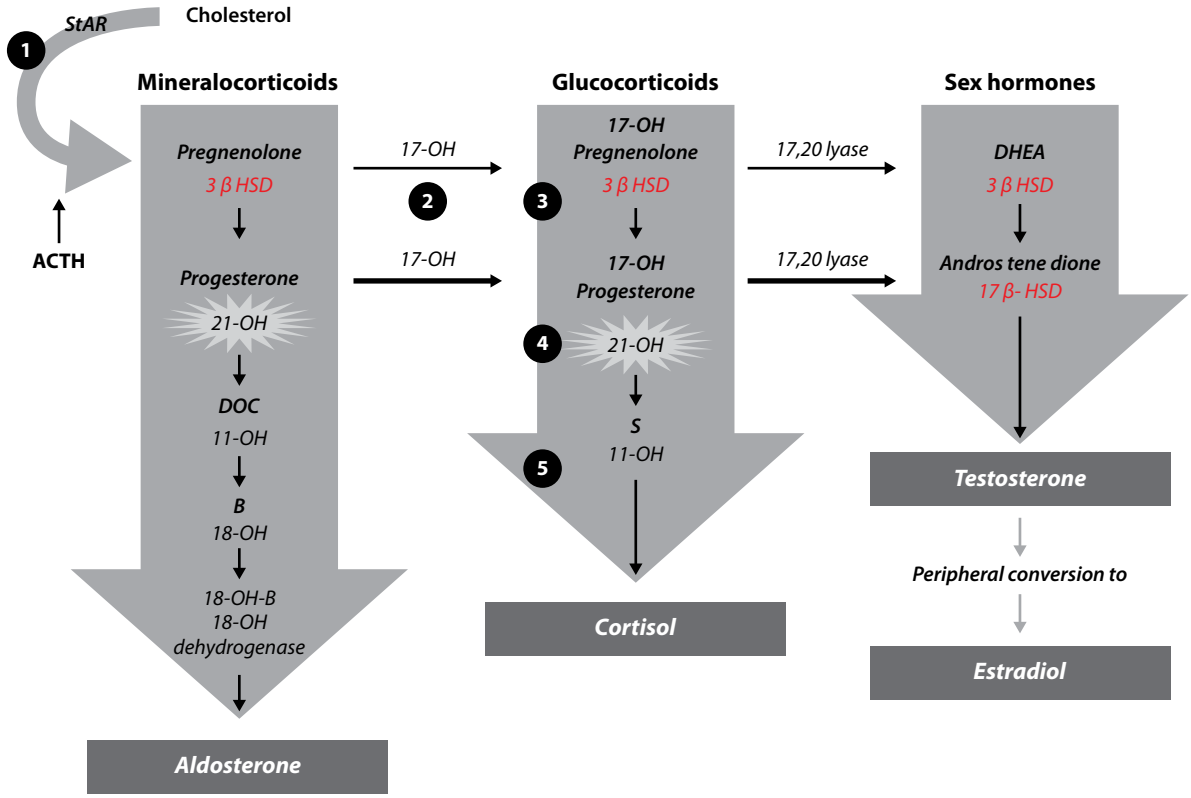

Figure 2.20. Adrenal steroidogenesis: Five enzymatic steps necessary for cortisol production are shown in numbers. 1= 20, 22 desmolase, 2= 17 hydroxylase (17-OH), 3=3ß-hydroxysteroid dehydrogenase (3ß HSD), 4=21 hydroxylase (21-OH), 5=11ß hydroxylase (11-OH). In the first step of adrenal steroidogenesis, cholesterol enters mitochondria via a carrier protein called StAR. ACTH stimulates cholesterol cleavage, the rate limiting step of adrenal steroidogenesis. (Figure from Lekarev et al. (2012); Used with publisher's permission).

the endoplasmic reticulum, most of which belong to the cytochrome P450 (CYP) family (**Figure 2.20**). The transfer of cholesterol across the mitochondrial membranes is facilitated by steroidogenic acute regulatory (StAR) protein. At the inner mitochondrial membrane, the cholesterol side chain is cleaved off, yielding pregnenolone. Pregnenolone is further converted to deoxycortisol, deoxycorticosterone, dehydroepiandrosterone (DHEA), and androstenedione. DHEA and androstenedione are weak androgens. Deoxycortisol and deoxycorticosterone are hydroxylated by the enzyme CYP11B1 to form the glucocorticoids cortisol and corticosterone. Deoxycorticosterone may also be transformed to the mineralocorticoid aldosterone.

In humans, cortisol and aldosterone are the physiologically most important hormones, while the androgen DHEA is the most abundant.

Glucocorticoids

Cortisol and corticosterone act to degrade protein and fat for use in gluconeogenesis with the aim to maintain blood glucose levels. The glucocorticoids are also involved in regulating blood volume and blood pressure and mediate anti-inflammatory and immunosuppressive effects. Glucocorticoids produced during embryogenesis by an adrenal progenitor influence the development of the neural crest-cells into what will become the adrenal medulla (Hammer, Parker & Schimmer, 2005).

Cortisol production is regulated through the hypothalamic-pituitary-adrenal (HPA) axis. In response to physical and/or emotional stress, the hypothalamus secretes corticotropin-releasing hormone (CRH), which causes the release of ACTH from the pituitary gland. ACTH stimulates transcription of several steroidogenic genes.

Mineralocorticoids

Aldosterone regulates water and electrolyte balance in the body by reducing excretion of sodium ions from the body, mainly by stimulating their reabsorption in the kidney. Hyper-secretion of aldosterone causes hypertension and retention of body fluid (edema) due to excess sodium and water retention. It also induces neuromuscular dysfunction due to loss of potassium.

Sex steroids

The hormone DHEA is produced in early fetal life (Hammer, Parker & Schimmer, 2005) by an adrenal gonadal progenitor. This hormone is essential for sustaining pregnancy before placental estrogens take over. The role of sex steroids produced by the adrenal cortex after birth is unclear. They are produced as early as 3 years of age in humans (adrenarche) and then gradually decrease after 30 years of age (adrenopause) (Kempná and Fluck, 2008; Idkowiak et al., 2011). They may cause pre-pubertal growth of pubic and axillary hair, and are possibly involved in the onset of puberty.

Steroidogenesis in the fetal adrenals

In humans, the fetal adrenal cortex is arranged differently than in adults (Coulter, 2005). The inner fetal zone comprises 85-90% of the entire gland and is producing DHEA throughout gestation. The outer definitive zone is inactive until late gestation, while the transitional zone is capable of producing glucocorticoids at the beginning of the third trimester. After birth, the fetal zone regresses. Fetal adrenal growth is independent of ACTH before 15 weeks of gestation, while after 15 weeks, ACTH and other factors are required for normal development (Langlois, Li & Sacz, 2002). Sensitive windows of human adrenal development are found between weeks 4 and 16 of pregnancy and comprise organogenesis from weeks 4 to 8 and establishment of steroidogenesis between weeks 12 and 16. The size of the new born adrenal is similar to its adult size (Langlois, Li & Sacz, 2002).

In the fetus, cortisol and corticosterone play an important role in the maturation of the organs, preparing them for an extra-uterine life and in induction of birth (Challis et al., 2001). In many mammals, such as ruminants and humans, there is a steep increase in cortisol that induces labour through activating enzymes such as CYP17 in the placenta (Wood, 1999).

Towards term, the HPA axis becomes progressively more responsive to stress stimuli (Fowden, Giussani & Forhead, 2005). Since glucocorticoids are responsible for maturation of tissues essential for neonatal survival, disruption of normal HPA axis activity may have widespread consequences. In humans, low birth weight is associated with elevated cortisol and aldosterone levels, suggesting fetal mis-programming of the HPA axis (Martinez-Aguayo et al., 2011)

2.8.2 Evidence for endocrine disruptor causation of adrenal hormone signalling in humans and in rodent models

EDCs may disrupt regulation of adrenal hormone secretion and function at different levels of the (HPA) axis. In addition to a targeted local toxicity in the hormone-secreting cells leading to mitochondrial degeneration and cell death, reduced hormone secretion may result from inhibition of steroidogenic (CYP) enzyme catalytic activity. Hormone secretion may also be up-regulated, both as a compensatory response from the HPA axis (increased ACTH secretion) or as a local response in the steroid-producing cells. Several persistent environmental pollutants are further known to reduce hormone secretion by inhibiting steroidogenic enzymes in vitro, while others induce mRNA synthesis and hormone secretion. Despite emerging evidence that adrenal function may be an important target for EDCs, only a limited number of studies have been published on EDC-induced disruption of adrenal hormone secretion in humans. Likewise, the numbers of experimental in vivo studies are few. However, a comparatively large number of in vitro studies based on the human adrenocortical cell line H295R have been published.

2.8.2.1 Humans

The information on EDC-induced effects on adrenal function in humans is restricted. No evidence is available to show that adrenocorticolytic POPs can affect the human adrenal cortex and HPA axis at environmentally relevant concentrations in vivo. However, there is evidence in the literature that environmental factors, including tobacco smoking and intake of alcohol, may affect the HPA axis and the way individuals respond to stress stimuli. For example, it has been reported that urban psychosocial stress may affect plasma cortisol concentrations (Rosati et al., 2011). Lessened activity of the HPA axis at the onset of and during a stress procedure was reported in adolescents who began drinking alcohol at an early age (Evans et al., 2012). Recent evidence also suggests that tobacco smoke exposure affects the activity of the HPA axis (Granger et al., 2007, Soldin et al., 2011). However, there is conflicting evidence as to what extent exposure to products of tobacco in utero can cause neurobiological and behavioural changes in exposed offspring (Huijbregts et al., 2011, Granger et al., 2007). Current evidence also suggests that reactivity to stress is reduced in cannabis users relative to abstainers (van Leeuwen et al., 2011).

2.8.2.2 In vitro studies

POPs present in human milk and adipose tissue may alter steroidogenesis and gene expression in the human H295R adrenal cell line in vitro. Both non-ortho and ortho-PCBs exert effects on steroidogenesis in H295R cells (Li & Wang, 2005; Xu et al., 2006). Both congener groups stimulated the steroidogenic machinery, although the potency of the different ortho-PCBs varied (Xu et al., 2006). Only small effects on hormone levels were measured after exposure to various hydroxylated PBDE congeners (Song et al., 2008), whereas in another study on PBDE metabolites greater increases in both estradiol and testosterone were observed, and expression of most steroidogenic genes were up-regulated (He et al., 2008). In contrast, the organohalogen pesticide γ-HCH (Lindane) decreased cortisol production in both stimulated and non-stimulated H295R cells (Oskarsson et al., 2006; Ullerås, Ohlsson & Oskarsson, 2008). Following prolonged exposure, $MeSO_2$-DDE decreases H295R cell viability in vitro (Asp et al., 2010b).

Complex mixtures of chemicals extracted from sediments, coastal waters, fresh water and sewage effluents have also been examined and reported to interfere with expression of steroidogenic genes in H295R cells (Blaha et al., 2006; Gracia et al., 2008, Zimmer et al., 2011a; Montano et al., 2011). There was not a uniform pattern of up- or down-regulation, but the CYP11B2 gene, encoding the enzyme that catalyzes the last step of aldosterone synthesis, was up-regulated by the majority of extracts tested. Data analysis performed on extracts from a Norwegian freshwater system suggested that PCBs, and to a lesser extent DDTs, were responsible for the cortisol responses, whereas estradiol and testosterone alterations were best explained by HCB and PCBs, respectively. Brominated flame

retardants were less important contributors to the steroidogenic responses (Zimmer et al., 2011a).

Recently, there has been a concern regarding the potential risk linked to intake of marine fisheries products and whether this risk is outweighed by the potential benefits. Exposure to extracts from crude cod liver oil was done in the H295R-model, resulting in effects on gene expression and hormone production similar to those induced by PCBs, which were major contaminants in the extracts (Montano et al., 2011). Observed effects after exposure to pharmaceutical oil extract, from which POPs had been removed, were considerably lower.

There is also increasing concern about exposure to and potential harmful effects of perfluorinated compounds on humans and the environment (see Chapter 3.1, 3.2.1 & 3.2.2 for exposure). These compounds are used in a huge number of industrial products and have attracted interest because of their persistence in the environment (Chapter 3.1.1). Recently published data using the H295R model provide some evidence that the adrenal cortex is a potential target for perfluorononanoic acid (PFNA) (Kraugerud et al., 2011). In a follow-up study PFNA reduced concentrations of testosterone, progesterone and cortisol.

2.8.2.3 In vivo studies with mammalian models

Some in vivo studies on adrenocorticolytic DDT metabolites and other compounds have been discussed in section 2.8.1 above. Other laboratory studies also give support to the contention that PCBs can also perturb adrenocortical function in mammals.

Some early studies in laboratory strains of mice and rats revealed effects of technical PCB mixtures on the HPA axis and cortisol homeostasis (Miller et al., 1993; Sanders & Kirkpatrick, 1975). Later $MeSO_2$-PCBs were found to be glucocorticoid receptor antagonists (Johansson, Nilsson & Lund, 1998), but so far no studies to explore the toxicological significance of this observation have been carried out. However, a recent developmental study in sheep does give support to the hypothesis that mono - and diortho PCBs are able to interfere with fetal adrenal development and cortisol production in mammals (Zimmer et al., 2011b). The results indicated that both CB-153 and CB-118 were associated with a decrease in fetal adrenal cortex thickness and decreased fetal plasma cortisol concentrations, whereas CB-118 also increased fetal ACTH. In adrenal cortex tissue, the expression of several genes encoding enzymes and receptors related to steroid hormone synthesis were down-regulated by PCBs. In addition, persistent effects of perinatal exposure to PCBs (CB-153 and CB-126) were indicated by the fact that intrauterine and lactational exposure affected the cortisol response to short-term stress in adulthood (Zimmer et al., 2009). Male goat kids exposed to either PCB congener showed a greater and more prolonged rise in plasma cortisol levels than controls when animals were subjected to mild stress at 9 months of age. However, neither the basal cortisol plasma level nor the adrenal mass were affected by PCB exposure.

2.8.3 Evidence for endocrine disruptor causation of adrenal hormone signalling in wildlife

2.8.3.1 Marine mammals

The most conclusive evidence for a causal link between adrenocortical disorders and exposure to persistent EDCs comes from studies in grey and ringed seals in the Baltic Sea (Bergman & Olsson, 1985). The high body burdens of PCBs and DDT compounds determined in the tissues of Baltic seals were associated with dramatic population declines that seemed to be most severe in the mid 1970s. The finding of a high incidence of occlusions/strictures in the uterine horns suggested that pregnancies were discontinued by early abortion of fetuses and that the subsequent development of strictures prevented new pregnancies. A high incidence of benign smooth muscle uterine tumours (leiomyoma) could also contribute to reproductive failure. In addition to these alterations in the female reproductive tract, a massive hyperplasia of the adrenal cortex was recorded in both sexes. Adrenocortical hyperplasia and a suite of alterations characteristic for Cushing disease were considered a cardinal finding in the disease syndrome (**Figure 2.21**). These alterations included decreased epidermal thickness, intestinal ulcers, and an osteoporosis-like condition (Bergman & Olsson, 1985; Lind et al., 2003). Although these alterations are compatible with adrenocortical hyper-secretion, no information on the plasma cortisol levels in these animals is available, and the exact mechanisms behind the Cushing-like condition remain unclear. Likewise, the individual compounds producing these adrenal lesions remain unknown. Although it cannot be ruled out that a component of stress was playing a role, it seems likely that persistent exposure to organohalogens were involved. Several adrenocorticolytic compounds are

Figure 2.21. Adrenocortical hyperplasia in grey seal (Bergman & Olsson, 1985). Transected adrenals from seals collected at Svalbard (left) and in the Baltic Sea (right). Note massive hyperplasia in the adrenal cortex (light area) of the Baltic seal. Dark area in both adrenals represents the adrenal medulla. (Photo: Anders Bergman).

known to accumulate in the adrenals and other tissues in seals (see Chapter 3.2.1). The temporal trends for reduced exposure to PCBs and DDT, and for recovery of the seals, support the conclusion that these POPs and their persistent metabolites were the causative agents. Along with the dramatic reduction of POPs in Baltic biota that has occurred during the last decades (Chapter 3.2.1.4), the Baltic seals have gradually recovered, supporting a role for classical legacy POPs in the etiology of this syndrome.

It is noteworthy that adrenocortical hyperplasia and Cushing-like alterations have not been reported in seal populations outside the Baltic Sea. Similar symptoms were, however, found in beluga whales. The adrenocortical lesions might have been caused by stress or by adrenocorticolytic xenobiotics, although such lesions could also be part of a normal ageing process (Lair et al., 1997).

In polar bears, which are among the most highly organochlorine-contaminated species of Arctic mammals, there is growing concern that several organochlorines (OCs) may be able to change basic endocrine pathways. Epidemiological data from 151 free ranging polar bears in the Svalbard region of the Arctic showed that alteration in the HPA axis, as indicated by plasma cortisol upon capture, was associated with plasma concentrations of OCs (Oskam et al., 2004). After correction for other factors, the overall contribution of OCs to plasma cortisol was negative, explaining around 25% of the variation in cortisol concentration. The plasma concentrations measured were comparable with plasma cortisol concentrations reported in experimental studies, in which ACTH was used to stimulate the adrenal cortex in order to produce a maximal cortisol response, indicating an activation of the HPA axis as a result of stress. In view of the complexity and lack of knowledge on stress responses in polar bears and their interactions with environmental factors, including OCs, it is difficult to predict the biological implications of such findings. However, these results, and the increasing evidence of EDC exposure effects during perinatal life stages, justify further attention to the effects of environmental contaminants on the HPA axis in polar bears and other highly exposed mammals.

2.8.3.2 Birds and amphibians

Both the p,p'-DDT metabolite, $MeSO_2$-DDE, and o,p′-DDD are known to be activated to toxic metabolites in the adrenal inter-renal cells of embryos and newly hatched chicks, implying that wild bird species should also be sensitive to adrenocorticolytic DDT metabolites (Jönsson el al., 1994). Although no information on such effects in wild birds is available as yet, several reports indicate that stress-induced responses of the HPA axis may be modulated by various environmental contaminants. High concentrations of organochlorines, brominated flame retardants and metabolism-derived products in blood plasma have been associated with high baseline corticosterone concentrations, and a reduced stress response, in arctic birds (Verboven et al., 2010). In a study on free-living, nestling white storks, Baos et al. (2006) reported that stress-induced corticosterone levels were positively correlated with the levels of lead in blood. In another study in mercury-contaminated tree swallow nestlings, stress-induced corticosterone levels were suppressed at the end of the nestling period (Wada et al., 2009). Studying pesticide-induced responses on the HPA axis in tree swallows and Eastern bluebirds, Mayne et al. (2004) observed a significant negative correlation between DDE levels and the capacity of bluebird chicks to elevate blood corticosterone levels in response to ACTH. Disruption of the responsiveness of the HPA axis has also been demonstrated in the common mudpuppy, an amphibian species, exposed to persistent organochlorines in the St Lawrence and Ottawa Rivers in Canada (Gendron et al., 1997). Altogether, these observations imply complex interactions of environmental pollutants with the HPA axis.

2.8.3.3 Fish

As is the case in mammals and birds, *o,p′*-DDD and $MeSO_2$-DDE are activated to toxic metabolites in the cortisol-producing adrenal inter-renal cells in various fish species (e.g. Lacroix & Hontela, 2003; Lindhe et al., 2003). Furthermore, deviation in cortisol levels and cortisol response to stress has been demonstrated in wild fish from polluted environments (Hontela, 1998) as well as in experimentally exposed fish (e.g. Jörgensen et al., 2002). Yellow perch and northern pike collected in PAH, PCB and heavy metal contaminated waters showed a reduced cortisol secretion in response to acute handling stress (Hontela et al., 1992; 1995). Combined with the observation that the ACTH-secreting cells in the pituitary were atrophic, it was proposed that life long exposure to contaminants resulted in exhaustion of the cortisol regulatory system in these fish. Studying the combined effect of PCB exposure and nutritional status in Arctic char, Jörgensen et al. (2002) observed that cortisol levels were elevated in food-deprived control fish compared with control fish given food during the winter period. The basal cortisol levels were decreased by PCBs in food-deprived fish but elevated in fed fish. It was proposed that the stress response in Arctic char is compromised by PCBs and that long term fasting, typical of high-latitude fish, make these fish particularly sensitive to persistent organochlorines.

The Norwegian Lake Mjøsa has been subject to major pollution from industry and agriculture in the past decades. High levels of PBDEs, predominately BDE -47 and -99 (approximately 450 times higher than concentrations measured in fish from un polluted lakes) have been found in fish from Lake Mjøsa together with significant amounts of PCBs, DDT and its metabolites. High concentrations of PBDEs were also reported in a group of high consumers of fish from Lake Mjøsa (Thomsen et al., 2008). Little evidence is available on effects caused by these compounds on wild fish or humans consuming fish from the lake. However, developmental and reproductive effects of lifelong exposure to environmentaly relevant concentrations of two natural mixtures of persistent

organic pollutants found in the lake were investigated using classical and molecular methods in a controlled zebrafish model (Nourizadeh-Lillabadi et al., 2009; Lyche et al., 2010; Berg et al., 2011; Lyche et al., 2011). Phenotypic effects observed in both exposure groups included earlier onset of puberty, increased male/female sex ratio, and differences in body weight at 5 months of age. Interestingly, genome-wide transcription profiling identified functional networks of genes, in which key regulators of weight homeostasis (PPARs, glucocorticoids, CEBPs, estradiol), steroid hormone functions (glucocorticoids, estradiol, NCOA3) and insulin signaling (HNF4A, CEBPs, PPARG) occupied central positions. The exposure effects found during development in zebrafish and the regulation of genes associated with weight homeostasis and insulin signaling suggest that environmental pollution may affect the endocrine regulation of body functions influenced by hormones and that the adrenal axis may play an important role.

2.8.4 Evidence for a common mechanism for adrenal disruption in humans and wildlife

A species comparison of the adrenocorticolytic DDT metabolites DDD and methylsulfonyl-DDE characterized in experimental systems suggests that these compounds should be considered as adrenal EDCs also in humans:

o,p'-DDD and *p,p'*-DDD

o,p´-DDD (Mitotane) and the *o,p´*-DDT as well as the environmentally more prevalent *p,p´*-DDD are formed by dechlorination of the corresponding DDT isomers. *o,p´*-DDD is a registered human pharmaceutical (Lysodren) for treatment of adrenocortical carcinoma (ACC) and Cushing disease in Europe and the USA. At therapeutic doses, which are high compared to environmental exposures, *o,p´*-DDD treatment results in reduced cortisol plasma concentrations and a reduction of adrenal tissue mass. The mechanism of adrenocorticolytic action has been worked out using adrenal tissue homogenate from several species including humans. *o,p´*-DDD is bioactivated to a reactive metabolite in a CYP-catalysed reaction which subsequently generates irreversibly bound protein adducts, mainly in the mitochondrial fraction of adrenal homogenate (Cai et al., 1995; Martz & Straw 1977; 1980). In dogs and mink, as in humans, *o,p'*-DDD induces cell death in the adrenal cortex in vivo and inhibits steroid hormone secretion in vitro (Hart, Reagan & Adamson, 1973; Jönsson, Lund & Brandt, 1993; Nelson & Woodard, 1949). The adrenocorticolytic activity is not restricted to the *o,p*'-DDD isomer but also resides in the environmentally more abundant *p,p´*-DDD isomer. The toxic potency of *p,p´*-DDD is roughly similar to that of *o,p´*-DDD in mink adrenal cortex in vivo and in human H295R cells in vitro (Asp et al., 2010a; Jönsson, Lund & Brandt, 1993).

Methylsulfonyl-DDE

As already mentioned, the second adrenocorticolytic DDT metabolite, $MeSO_2$-DDE, was originally identified in blubber of Baltic grey seal (Jensen & Jansson, 1976), a species suffering from adrenocortical hyperplasia. There is strong evidence to suggest that humans are sensitive to this compound, demonstrated to be a highly potent and completely tissue-specific adrenal toxicant activated by the enzyme CYP11B1 (11ß-hydroxylase) in the *zona fasciculata* of fetal (**Figure 2.22**) and adult mice (Lund, Bergman & Brandt, 1988; Jönsson et al., 1992; Jönsson, 1994; Jönsson, Rodriguez-Martinez & Brandt, 1995). CYP-catalysed irreversible binding of $MeSO_2$-DDE has been confirmed both in human adrenal homogenate (Jönsson & Lund, 1994), and in human adrenal tissue slice culture (Lindhe, Skogseid & Brandt, 2002). Moreover, $MeSO_2$-DDE alters glucocorticoid synthesis in human adrenal tissue slices ex vivo (Lindhe, Skogseid & Brandt, 2002) and in the human adrenal cell line H295R in vitro (Asp et al., 2010b).

2.8.5 Main messages

- The adrenal cortex has been identified as the most commonly affected and vulnerable endocrine organ in toxicology. Experimental data and data from exposed wildlife populations suggest that both the HPA axis and the adrenal glands are targets for endocrine disruption caused by pollutants at environmentally relevant exposure concentrations. Despite this fact, and compared with other

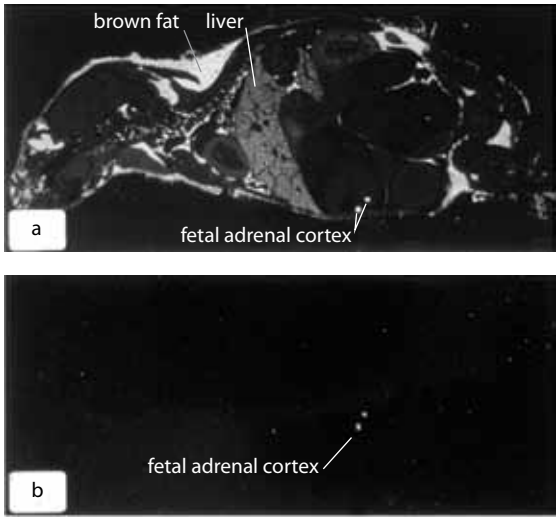

Figure 2.22. Autoradiograms showing the tissue-specific irreversible binding of ^{14}C-labelled $MeSO_2$-DDE in the fetal adrenal cortex of a pregnant mouse (Jönsson et al., 1992). The autoradiogram in the lower panel reveals that an irreversibly bound adduct is confined to the fetal adrenal cortex (maternal adrenals not present), while all other tissues are devoid of bound adducts. The upper panel shows the distribution of both the unmetabolized $MeSO_2$-DDE (high concentrations in fat and liver) and its covalently bound adduct (fetal adrenal cortex). White areas in the autoradiograms correspond to high levels of radioactive compound. (Figure reproduced with publisher's permission)

endocrine axes, the HPA axis has so far gained relatively little attention in endocrine disruptor research.

- The behavioural and physiological traits of an individual are strongly influenced by events occurring during embryonic/ fetal life. The HPA axis is one of the major systems implicated in the responses to environmental manipulations and stress. Glucocorticoid hormones represent the final step in the activation of the HPA axis and play an important role in effects induced by the perinatal environment.
- Developing organs are particularly sensitive to alterations in hormone levels, and exposure to chemicals during critical windows of development may cause effects on the adrenal function that persist until adulthood. Recent experimental data suggest that environmentally relevant exposures to pollutants (PCBs) affect development of the fetal adrenal cortex, the function of the HPA axis, and induce delayed effects in the response to stress in animal models.
- A disrupted HPA axis may lead to altered stress responses and changes in cognitive functions. A topic for further research would be to investigate whether selected POPs and their mixtures will affect endocrine cells in the HPA axis following relevant exposures during early life-stages, and whether these changes will result in long-term effects on cognitive functions and stress responses.

2.8.6 Scientific progress since 2002

Adrenal dysfunction was not fully covered in the 2002 *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002).

2.8.7 Strength of evidence

There is sufficient evidence to show that adrenocortical hyperplasia and a suite of pathological changes characteristic of Cushing disease in Baltic seals were caused by exposure to a mixture of DDT, PCBs and their methylsulfonyl metabolites; along with the drastic reduction of DDT and PCBs in Baltic biota, the seal populations have gradually recovered.

There is sufficient evidence to show that adrenocorticolytic DDT metabolites have a similar mechanism/mode of action in a variety of vertebrate species including in human adrenal tissues and cells.

There is some evidence to show that altered responsiveness of the HPA axis is associated with tobacco smoking and alcohol consumption in human subjects, and with exposure to persistent environmental pollutants and toxic metals in wild mammals, birds and fish and that PCBs may disrupt the feedback system regulating cortisol levels in young and adult sheep and goats.

For the great majority of chemicals in modern commerce, there is no evidence to show effects of exposures on adrenal function. Nor have there been any studies to test this hypothesis. There is sufficient evidence to suggest that a variety of chemicals and mixtures alter gene expression and hormone synthesis in the human adrenal H295R cell line in vitro.

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2.9 Endocrine disrupting chemicals and bone disorders

2.9.1 Overview of bone disorders in humans and wildlife and evidence for endocrine disruption

Since World War II, there has been an increase in age-standardized incidence rates of osteoporotic bone fractures in industrialized countries, with the Nordic countries taking the lead (Ismail et al., 2002). The reason for this increase is unknown, but the idea that exposure to EDCs could be involved has been suggested (Lind, 2000) with the hypothesis that EDCs affect bone, as well as reproductive tissues, and that these effects are sex-dependent. If this hypothesis is proven, exposure to EDCs could have contributed to the increase in the observed age-standardized incidence rates of osteoporotic fractures observed in most industrialized countries, mainly in women (Kanis et al., 2012). The literature on this topic is, however, rather small.

2.9.1.1 The role of hormones in bone formation

It is well established that bone is a target tissue for estrogens; both ERα and ERß have been identified in bone cells (Eriksen et al., 1988; Onoe et al., 1997; Migliaccio et al., 1992a) and several studies have revealed that estrogens can influence bone cells in vitro by a direct ER-mediated mechanism (e.g. Migliaccio et al., 1992b; Oursler et al., 1993). Moreover, intrauterine exposure to the endocrine disruptor diethylstilbestrol increased bone mass, but decreased bone length in adult female offspring (Migliaccio et al., 1992b).

In women, a marked decrease in bone mineral density is seen following oophorectomy and bone loss is markedly increased also in the years following menopause, when the circulatory levels of estrogen are reduced (for reviews see Kanis 1996 and Francucci et al., 2010). Hormone replacement therapy with estrogens decreases the rate of further bone loss with age, thus providing corroboration of the relationship between bone mineral density and estrogen (Lindsay et al., 1980). Furthermore, mutations in genes encoding for the estrogen receptors in a young man were reported to result in tall stature, delayed bone maturation and development of severe osteoporosis. The authors concluded, therefore, that estrogen is important for bone maturation and mineralization in men as well as in women (Smith et al., 1994). This observation was subsequently supported by the finding that estrogen deficiency is involved in the pathogenesis of osteoporosis in men (Khosla et al., 1998).

2.9.2 Evidence for endocrine disruptor causation of bone disorders in humans and in rodent models

Epidemiology

At the end of the 1950s, in Turkey, more than 4000 people were accidentally poisoned due to ingestion of hexachlorobenzene (HCB) that had been added to wheat seedlings as a fungicide. The exposed individuals displayed a huge spectrum of symptoms, such as dermatological, neurological and orthopedic defects. In observations performed 20-30 years after the accident, it was revealed that as many as 64.7% of the victims had resorption of digits due to osteoporosis (Cripps et al., 1984).

In another food poisoning accident, several thousands of people in Japan (Yusho) ingested rice-oil contaminated with PCBs and polychlorinated dibenzofurans (PCDFs). In this accident too, the victims exhibited an extensive range of symptoms: children born to exposed mothers were shorter and skeletal lesions, such as irregular calcification of the skull bones and dentition at birth, were also found (Miller, 1985; Yamashita & Hayashi, 1985).

Two rather small cross-sectional population-based studies also demonstrated a negative relationship between the levels of the DDT metabolite, *p,p´*-DDE, and bone density (Beard et al., 2000; Glynn et al., 2000). Furthermore, it has been shown that Swedish fishermen's wives on the east coast who ingested fatty fish from the Baltic Sea had a significantly increased incidence of osteoporotic and hospitalized vertebral fractures when compared to fishermen's wives eating fish from the Swedish west coast (Wallin, Rylander & Hagmar, 2004); the high dietary intake of POPs through fatty fish might be a risk factor for vertebral fractures, because the levels of POPs have been shown to be much higher in fatty fish from the Baltic Sea compared to the Swedish west coast.

Evidence from laboratory studies that EDCs could impair bone structure and function

Experimental studies with laboratory rodents in vivo and with bone tissues in vitro suggest that bone tissue could be an important target for a number of endocrine disrupting persistent organohalogen pollutants, including hexachlorobenzene (Andrews et al., 1989), PCBs (Lind et al., 1999; Lind, 2000; 2004a; Lundberg et al., 2006; Alvarez-Lloret et al., 2009; Gutleb et al., 2010), and high affinity AhR-ligands such as TCDD (Jämsa et al., 2001; Miettinen et al., 2005; Wejheden et al., 2006; Hermsen et al., 2008), since exposure to those compounds impaired both structure and function of bone tissue in all of these studies.

2.9.3 Evidence for endocrine disruptor causation of bone disorders in wildlife

The possibility that EDCs might contribute to bone disorders in humans is supported by a range of studies in wildlife, including those in East Greenland polar bears (Sonne et al., 2004), Baltic grey seals (Lind et al., 2003) (See **Figure 2.23**), American alligators (Lind et al., 2004b), clapper rails (Rodrigues-Navarro et al., 2006), and herring gulls (Fox et al., 2008). In all cases, bone tissue abnormalities were correlated with exposure to mixtures of pollutants.

Figure 2.23. Skull of 14 year old male Baltic grey seal showing loss of bone and several teeth but also exostoses (outgrowth, to the right in the picture) on maxillary bone, found 1990 at the Baltic coast. (Photo: Hans Lind)

2.9.4 Main messages

- Very limited studies, published mostly within the last few decades, indicate that bone tissues of experimental and wild animals are negatively affected by exposure to persistent EDCs.
- Epidemiological studies on humans also support this hypothesis since they show a relationship between exposure to endocrine disrupting POPs and decreased bone mineral density or increased risk of bone fractures.
- Prospective studies on *in utero* or early EDC exposure and future osteoporotic bone fractures are missing and the mechanisms behind the deleterious effects of EDCs on bone tissue also need to be further studied.

2.9.5 Scientific progress since 2002

Bone disorders were not covered in the 2002 *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002).

2.9.6 Strength of evidence

There is some evidence to show that accidental poisoning of humans with persistent organic pollutants HCB, PCBs and DDT that are also endocrine disruptors caused bone disorders, and a plausible, although not proven, endocrine mechanism of action through which these disorders may have occurred.

For the great majority of chemicals in modern commerce, there is no evidence to show effects of exposures on bone function. Nor have there been any studies to test this hypothesis.

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2.10 Endocrine disruptors and metabolic disorders

2.10.1 Overview of metabolic disorders in humans and wildlife and evidence for endocrine disruption

The role of hormones and of endocrine disruptors in metabolic disorders such as obesity and diabetes has been a topic of recent research. There are multiple reasons for this:

- Metabolic disorders are continuing to rise in human populations, including in children and adolescents. Established risk factors alone cannot account for these disease trends.
- Some endocrine disrupting chemicals can affect the function of the insulin producing beta cells in the pancreas (e.g. Cooper et al., 2009) and are also immunotoxic.
- There are now animal data suggesting that exposure to some endocrine disrupting chemicals during pregnancy can lead to altered cholesterol metabolism, weight gain and type 2 diabetes in the offspring later in life.

This section of our review considers the evidence for the involvement of endocrine disrupting chemicals in causing metabolic disorders.

2.10.2 Evidence for endocrine disruptor causation of metabolic disorders in humans and in rodent and primate models

2.10.2.1 Obesity

Secular trends

The prevalence of obesity has risen dramatically in many parts of the world over the past two decades, as in the OECD countries (Sassi et al., 2009; OECD 2010) graphically presented in **Figure 2.24**. In the United States, for example, some reports cite 30% of adults as clinically obese and 65% as overweight (Ogden et al., 2007; Cunningham, 2010). Perhaps more important is that the rate of obesity and its related diseases, such as diabetes, are rising dramatically in children: estimates from the USA suggest that more than 60% of children 10 years of age and older either are currently obese or will become obese later in life (Oken & Gillman, 2003).

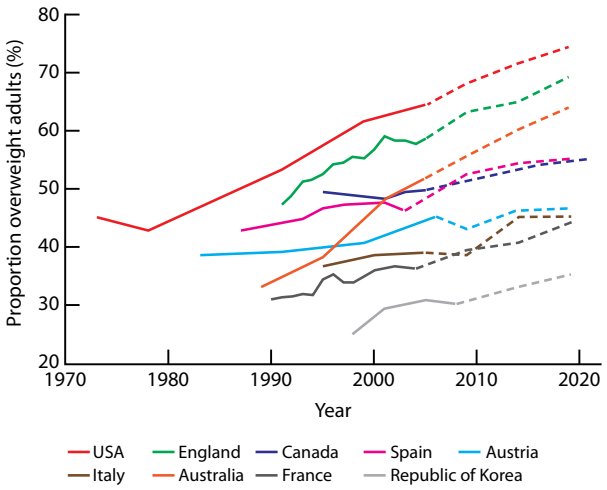

Figure 2.24. Past (solid lines) and projected (dashed lines) overweight rates in selected OECD countries (OECD, 2010). Used with publisher's permission.

There is evidence that the obesity risk may begin early in life, during pregnancy, and in early childhood and that rapid weight gain, in the first few months of life, is associated with obesity later in life (Ong et al., 2000; McAllister et al., 2009)

Endocrine role

Obesity is a complex endocrine-related disease caused by the interaction between genetic, behavioural, and environmental factors (Stanley et al., 2005). The control of weight gain is governed by many components of the endocrine system, including the adipose tissue, brain, skeletal muscle, liver, pancreas and gastrointestinal (GI) tract. Thus, there are multiple endocrine and paracrine factors that need to integrate to control this multi-functioning system and to regulate adipose development, number and function of fat cells, food intake, satiety, pleasure-related (hedonic) reward mechanisms, insulin sensitivity, lipid metabolism and, ultimately, body weight (Newbold et al., 2008; Grun & Blumberg, 2009). Estrogens, androgens, glucocorticoids, and thyroid hormones play important roles in controlling adipose tissue development, metabolism, and satiety.

Evidence for endocrine disruption

Because obesity is an endocrine-related disease/dysfunction, it is potentially sensitive to endocrine disrupting chemicals (Ropero et al., 2008; Sargis et al., 2010). In addition to the well-established modern societal influences of over-nutrition and lack of exercise, it has been hypothesized that exposures to chemicals are also contributing to the rapid rise in cases of obesity (Newbold et al., 2008; Newbold, 2010; Keith et al., 2006). Indeed, there are now data in animal studies indicating that chemical exposures during vulnerable windows of development may affect adult weight (Newbold, Padilla-Banks & Jefferson, 2006; Baillie-Hamilton, 2002). For instance, there are animal data suggesting that developmental exposure to chemicals including tributyltin, bisphenol A, organochlorine and organophosphate pesticides, air pollution, lead, diethylstilbestrol, perfluorooctanoic acid, monosodium glutamate and nicotine can lead to altered cholesterol metabolism and weight gain later in life (Newbold, Jefferson & Padilla Banks, 2007; Newbold et al., 2008; Grun et al., 2006; La Merrill & Birnbaum, 2011; Heindel & vom Saal, 2008; Li, Ycaza & Blumberg, 2011; Slotkin, 2011; Dirinck et al., 2011; Janesick & Blumberg, 2011; **Table 2.6**). Chapter 3.2.2) provides an overview of exposure to some of these chemicals. There are, however, limited data in humans (Tang-Peronard et al., 2011) supporting the notion that exposure to

such chemicals during development can affect weight gain in infants and in children (La Merrill & Birnbaum, 2011; Verhulst et al., 2009; Heindel, 2011). These epidemiology studies suffer from limited numbers of subjects, lack of long term follow up and examination of only single chemicals and single time points. The strongest epidemiology data show that smoking during pregnancy leads to weight gain later in life in the offspring. Indeed, a meta-analysis of over 15 studies showed a consistent effect of this factor (Oken, Levitan & Gillman, 2008).

Chemicals with endocrine disrupting properties may potentially act either on specific or multiple sites to:

- Alter endocrine pathways responsible for control of adipose tissue development
- Increase the number of fat cells
- Alter food intake and metabolism via effects on sexually dimorphic and appetite and reward centers in the brain
- Alter insulin sensitivity and lipid metabolism via effects on endocrine (and endocrine-related) tissues such as the pancreas, adipose tissue, liver, GI tract, brain and muscle

The net result of these changes is an alteration or deregulation of the "endocrine set point" or changes in homeostatic sensitivity that predisposes individuals to obesity later in life. Chemicals that have been shown to cause increased weight gain in animal models have been termed obesogens (**Table 2.7**). Chemicals that cause both weight gain and alter lipid metabolism and glucose sensitivity have also been called

Table 2.6. List of known and suspected environmental obesogens (A=Animal study, C=Cell culture study, H=Human study). Janesick & Blumberg (2011) provide more detailed information about obesogens.

Chemical	Commercial use	Relevant EDC action	Obesogenic activity
Tributyltin	Pesticide, wood preservation	Binds PPARγ	Changes identity of adipose precursors, increases triglycerides in adipose tissue (A)
Phthalates	Plasticizer	Binds PPARγ	Induce adipocyte differentiation (C), men's waist size (H)
PFOA	Non-stick coatings	Weakly activates PPARγ	Induce adipocyte differentiation (C))
Flavanone	Natural plant products used as flavourings	Binds PPARγ	Induce adipocyte differentiation (C))
PCBs	Electronics	Binds AhR in adipocytes	CB-77 promotes adipocyte differentiation, obesity (C,A)
Bisphenol A	Plastics	Binds ER, ERRγ	Induces adipogenesis (C), obesity (A)
Hexachlorobenzene	Fungicide	Alters TH signaling	Gestational exposure levels influence BMI (H)
Bisphenol A diglycid ether	Epoxy resins	Unknown	Induces adipogenesis (C)
PBDEs	Fire retardants	Reduces thyroid function	Stimulate fat production (C)
Diethylstilbestrol	Pharmaceutical estrogen	Binds ER	Perinatal exposures cause obesity (A). BMI in young children (H)
Genistein	Natural component in soy	Binds ER	Perinatal exposures cause obesity (A).
Perfluoroalkyl sulfonate	Non-stick coatings	Binds ER	Perinatal exposures cause obesity, alter insulin & leptin levels (A).
Nicotine	Found in tobacco products		Alters development of pancreas & adipose tissue, increases adipose cell size (A)
DDE	DDT metabolite	Binds ER	Concentrations in mothers associated with weight and BMI in female offspring (H)

The mechanisms by which most of these chemicals affect weight gain are largely unclear. Tributyltin, one of the few chemicals studied in detail, activates the combined peroxisome proliferator -activated receptor gamma (PPAR-γ)/ retinoid-X-receptor (RXR) pathway, the main pathway for fat cell differentiation (Janesick & Blumberg, 2011) and thereby stimulates fat cell differentiation in vitro and increases adipose tissue in vivo in mice. Similarly, chemicals with estrogenic activity like DES, genistein and BPA appear to act via estrogen receptors on fat cells, and cells of the brain and other tissues to regulate adipose tissue and food intake (Janesick & Blumberg, 2011).

Table 2.7. The obesogen hypothesis

During development: Environmental chemicals with endocrine activity
• Alter programming of components of the homeostatic or • Hedonic, reward, pathway and or • Number of fat cells and/or • Energy expenditure and/or • Inflammation/inflammatory responses and/or • Emotional and/or stress responses
Throughout life: Continued stress on an already abnormal metabolic system
• Increased access to high fat and sugar foods leading to "food addiction" • Lifestyle with reduced activity • Continued exposure to "obesogenic" chemicals leading to more fat cells, altered homeostatic and reward pathways

metabolic disruptors but also fit under the term obesogens. (Casals-Casas & Desvergne, 2011).

2.10.2.2 Type 2 diabetes

Type 2 diabetes (formerly called non-insulin-dependent or adult-onset) occurs when the body cannot effectively use the insulin produced by the pancreas. Insulin is a hormone that regulates blood sugar. Hyperglycemia, or raised blood sugar, is a common consequence of uncontrolled diabetes which can cause serious damage to many of the body's systems, especially the nerves and the blood vessels. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity.

Secular trends

Diabetes (including type 2 diabetes) poses a serious burden on public health systems around the world and is recognized as a global epidemic (Wild et al., 2004). Similar to obesity, estimates of the global prevalence of diabetes suggest it has more than doubled over the past three decades from 153 (127-182) million people in 1980 to 347 (314-382) million in 2008 (Danaei et al., 2011). This recent study of diabetes prevalence for adults aged 25 years and older in 199 countries and territories revealed that the worldwide age-standardized adult diabetes prevalence had risen to 9.8% (8.6-11.2) in men and 9.2% (8.0-10.5) in women, up from 8.3% (6.5-10.4) and 7.5% (5.8-9.6) in 1980. As important as the global rise are the trends in different countries, ranging from nearly flat in some regions to the steepest rise in age-standardized prevalence of 5.9 percentage points for men and 7.8 percentage points for women of Oceania (Marshall Islands, Samoa, Kiribati), to reach 21-25% of adult men and 21-32% of women in this region. Large increases were also recorded in southern and tropical Latin America, south and central Asia, North Africa, and the Middle East (Saudi Arabia). No change in diabetes prevalence was recorded in central and eastern Europe during these 28 years, although ageing and population growth led to an increase in the number of people with diabetes in these regions. Of the high-income countries studied, diabetes prevalence in 2008 was highest in the USA, Greenland, Malta, New Zealand, and Spain and lowest in The Netherlands and Austria. Over the preceding three decades, diabetes increased the least and the most in Western Europe and North America, respectively. Notwithstanding these comprehensive statistics, global estimates of diabetes are still far from complete even today; no population-based data were identified for 92 countries of the world, especially from some low-income and middle-income countries.

Seventy percent of risk associated with type 2 diabetes is attributed to excess weight gain, suggesting a direct metabolic link between increased adiposity and type 2 diabetes. For example, American statistics suggest that obesity is the leading cause of this disease (CDC, 2008). Of particular concern is the fact that the incidence of type 2 diabetes is increasing in children and adolescents at similar rates to the increases in obesity; data from North America show that one in four overweight children have impaired glucose tolerance. Type 2 diabetes is more common in girls than boys and girls are less insulin sensitive as early as 5 years of age (Pleis, Lucas & Ward, 2009). Notwithstanding the relationship between diabetes and obesity, global and regional trends in diabetes differ from those of other metabolic risks such as high blood pressure, which decreased globally and total cholesterol which decreased in Australasia, Europe, and North America, but rose in east and southeast Asia and Asia-Pacific, leading to relatively unchanged global means (discussed in Danaei et al., 2011). Because high BMI is a risk factor for all three metabolic indicators, the differences in diabetes trends are probably due to other factors; chief amongst these are diet and medical treatment.

Endocrine role

Maintenance of blood glucose homeostasis involves a series of complex gene-environment interactions between different tissues, including the liver, skeletal muscle, adipose tissue, brain and the pancreas. Altered glucose homeostasis leads to type 2 diabetes, which consists of defects in both insulin secretion and insulin action (insulin resistance) (Leng, Karlsson & Zierath, 2004), both of which are controlled by the endocrine system. Since the beta cells of the pancreas are central to controlling glucose homeostasis, an endocrine disrupting chemical that can initiate, facilitate and/or accelerate the loss of beta cell function can play an important role in type 2 diabetes. As already mentioned, obesity is the leading comorbidity factor in type 2 diabetes. Other contributing factors include increased tissue inflammation, reduced adiponectin secretion (a protein hormone produced exclusively by the fat cells that regulates metabolism of lipids and glucose), impaired inhibition of the breakdown of fat by the hormone insulin and altered liver function (Hevener & Febbraio, 2010). Type 2 diabetes is also closely related to thyroid function (see section 2.5) due to common signalling pathways and genetic susceptibility.

Evidence for endocrine disruption

There are limited data showing that, like obesity, sensitivity to develop type 2 diabetes is programmed during development and that both altered nutrition and exposures to environmental chemicals may be important in its etiology. Indeed numerous endocrine disrupting chemicals (EDCs) have been implicated in the development of type 2 diabetes, in both animal and epidemiological studies, including bisphenol A, phthalates, flame retardants, arsenic, POPs, and pesticides (reviewed by Alonso-Magdalena, Quesada & Nadal, 2011)

In humans, there is growing epidemiological evidence that adult exposures to EDCs may contribute to the development of type 2 diabetes: studies report an increased risk of type 2 diabetes after exposure to POPs (including PCBs, DDE, dioxin, organochlorine pesticides, hexachlorobenzene), arsenic and some flame retardants (e.g. Neel & Sargis, 2011; Everett, Frithsen & Player, 2011; Reilly et al., 2011; IPCS, 2011). For example, a 2006 study found a relationship between six POPs and diabetes in USA adults: the risk of type 2 diabetes was 37.7 times higher in people with the highest exposure than in

people with the lowest levels of exposure. The POPs included the dioxins HpCDD and OCDD, DDE, CB-153, oxychlordane, and trans-nonachlor, with the latter three showing the most significant relationships (Lee et al., 2006). Moreover, a nested case control study and a prospective study in adults both showed that exposure to a variety of POPs (PCBs and organochlorine pesticides) was associated with type 2 diabetes (Lee et al., 2011). A mechanism that links exposure to POPs with type 2 diabetes is unclear currently, but many of the POPs have endocrine disrupting activity, including effects on thyroid (see Chapter 3.1.1, 3.2.2.2 & 3.2.2.3 for an overview of human exposure to some of these chemicals).

Of the chemicals in modern commerce, Lang et al. (2008) reported that higher urinary concentrations of bisphenol A were associated with diabetes and altered liver function in USA adults in 2003- 2004 using the National Health and Nutrition Examination Survey (NHANES) dataset. In addition, some epidemiological studies have linked arsenic exposure to diabetes, especially in areas with high arsenic levels in drinking water, although whether lower exposure levels are also diabetogenic is unclear (Huang et al., 2011; Saldana et al., 2007). Arsenic exposure has been associated with impaired glucose tolerance in women with relatively low levels of arsenic exposure during pregnancy (Ettinger et al., 2009; see Chapter 3.1.1.8 & 3.1.5 for information on arsenic exposure).

Since gestational diabetes (a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy) increases the risk of later diabetes in the mother and the risk of later glucose abnormalities in the child, the role of endocrine disrupting environmental chemicals in gestational diabetes should be further investigated. The lack of longitudinal data, or developmental exposure data, on less persistent chemicals in modern commerce make it difficult to form conclusions from the existing data, without developmental exposure information.

In animal studies, exposures to environmental chemicals during critical developmental periods, such as in utero, have been linked to the later development of glucose intolerance and insulin resistance. For example, one study showed that in utero exposure to bisphenol A caused increased insulin resistance and secretion and glucose intolerance in adult male mice. Bisphenol A exposure to the pregnant mothers also decreased glucose tolerance and increased insulin resistance to the mothers during pregnancy, and increased insulin resistance 4 months post-partum (Alonso-Magdalena et al., 2010). Developmental exposure to arsenic and organophosphorous pesticides have also been linked to later diabetogenic effects in the offspring. Low level exposure to arsenic, from prenatal development until adulthood, leads to beta cell damage, impaired glucose tolerance, hyperglycaemia, increased insulin resistance, and altered insulin secretion in rats (Davila-Esqueda et al., 2011). Moreover, early post-natal exposure to organophosphorous pesticides produces lasting effects on metabolism that are consistent with pre-diabetes. Some effects intensified between adolescence and adulthood, while others waned (Adigun et al., 2010). Moreover, the pre-diabetic effects of exposure to these pesticides during critical windows of development have been shown to be exacerbated by a high-fat diet in adulthood (Slotkin, 2011). Taken together, these effects raise the possibility that exposure to chemicals during pregnancy may contribute not only to type 2 diabetes in the offspring, but also gestational diabetes and later type 2 diabetes in the mothers. The effect on insulin secretion has been specifically shown to involve estrogen receptors and a role for estrogen receptors in all of the effects of endocrine disruptors on the pancreas and adipose tissue is suspected (Toschke et al., 2003; Salsberry & Reagan, 2007; Karmaus, et al., 2009; Richter et al., 2007; Lim et al., 2009).

In adult animals, some chemicals can cause diabetes or insulin resistance following adult exposures. One analysis followed primates who were fed the pesticide DDT for 130 months and analysed at the age of 18-24 years. Two of the 24 exposed monkeys developed diabetes, and two developed hypoglycemia, compared to none in the control group. The exposed monkeys also developed fatty changes in the liver, central nervous system abnormalities, and tumours more often than controls (Takayama et al., 1999). In rodents also, pesticides and air pollutants have been shown to contribute to diabetes-related effects following adult exposures. For example, long term, low dose exposure to the current-use herbicide, atrazine (see Chapter 3.1.1.6) caused insulin resistance in adult rats, an effect worsened by a high-fat diet (Lim et al., 2009). Similarly, adult male rats exposed to fine particulate matter (PM2.5) in conjunction with a high fat diet developed insulin resistance (Yan et al., 2011). Adult male rats exposed to the POPs present in crude farmed Atlantic salmon oil developed insulin resistance and hepatosteatosis. In another study, the same POPs, especially the organochlorine pesticides, were shown to inhibit insulin action in cultured adipocytes (Ruzzin et al., 2010).

Some metals also have diabetogenic effects in adult rodents. For example, exposures to cadmium have high blood glucose levels and impaired glucose tolerance (Edwards & Prozialeck, 2009). It can also impair insulin secretion by beta cells as well as reduce glucose uptake by peripheral tissues, processes that likely contribute to diabetogenicity. Arsenic can also lead to higher blood glucose levels, higher insulin levels, and impaired glucose tolerance, depending on the dose, form of arsenic, and duration of exposure (Huang et al., 2011).

2.10.2.3 Type 1 diabetes

Secular trends

Type 1 diabetes (T1DM, IDDM, or formerly, juvenile diabetes) is a form of diabetes that results from selective autoimmune destruction of insulin-producing beta cells of the pancreas (for a full discussion of autoimmune diseases, see section 2.11). The consequent lack of insulin leads to increased blood and urine glucose. At clinical diagnosis there are usually only 10-20% remaining active beta cells. This clinical presentation is preceded by an asymptomatic period of high variability from months to many years or decades. Type 1 diabetes is the most common autoimmune-related disease in children. In recent decades, there has been a progressive decrease in the age of onset of type 1

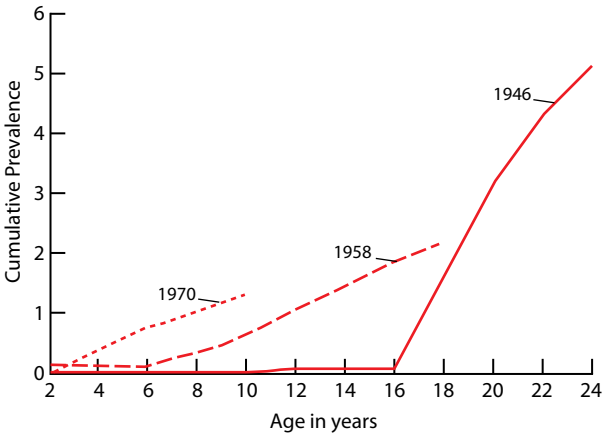

Figure 2.25. A progressive leftward shift in age of onset of childhood type 1 diabetes has been and continues to be observed in the United Kingdom. This trend in a progressively earlier onset of diabetes is consistent with data from other countries. In 1946 no diabetes was shown in children until 16 years of age; in 1970, at 2 years of age. Source: Diabetes, 2002 American Diabetes Association, Inc. www.medscape.com (Used with publisher's permission).

diabetes; the incidence has increased among children under the age of 15 yrs, with the biggest increase in children under 5 yrs old (Ziegler et al., 2011; **Figure 2.25**).

Evidence for endocrine disruption

Preliminary epidemiology studies support a role for exposure to chemicals in type 1 diabetes. While limited, there are epidemiological data linking adult exposures to nitrate/nitrite/nitroso compounds, air pollutants ozone and sulfate, and PCBs with increased incidence of type 1 diabetes (Howard & Lee, 2012)

It seems likely that type 1 diabetes, like obesity and type 2 diabetes, has its origins during development; in utero and/or the first few years of life. While there are no compelling animal data linking exposure to endocrine disruptors to the development of type 1 diabetes, there are chemicals noted above for type 2 diabetes that can affect beta cell function, including bisphenol A, PCBs, dioxin, arsenic, and phthalate plasticizers (see Chapter 3.1 and 3.2.2). It is interesting that many of these same chemicals (e.g. the estrogens bisphenol A, DES, chlordecone as well as dioxin, phthalates, and trichloroethylene) are also immunotoxic in animal models (e.g. Cooper et al., 2009 and see section 2.11). Thus a hypothesis that needs testing is that chemicals with both immunotoxic/autoimmune activity and also endocrine disrupting activity could act via both mechanisms to cause type 1 diabetes. The endocrine disrupting effects could alter the development of beta cells, and the immunotoxic component could lead to the production of auto-antibodies, resulting in the destruction of the altered and susceptible beta cells leading to type 1 diabetes.

2.10.2.4 Metabolic syndrome

Metabolic syndrome is also associated with the rise in obesity and may contribute to the progression of type 2 diabetes (Abrams & Levitt Katz 2011). Metabolic syndrome is defined clinically as a combination of at least three of the following five dysfunctions: hypertension, abdominal (central) adiposity, increased serum triglycerides and low serum high density lipoproteins (HDL) and high blood sugar, even after fasting (Steinbeck 2004).

There are significant data supporting the notion that disruptions of developmental programming cause metabolic syndrome and that there is a role for maternal diet in its aetiology (Ng et al., 2010). While there are currently no data linking developmental programming and environmental chemical exposure to metabolic syndrome *per se*, there are studies that have shown effects of chemical exposure on the progression of obesity and type-2 diabetes, noted above (Newbold, 2010; La Merrill & Birnbaum 2011) and a possible role of exposures to endocrine disrupting chemicals as a causative factor of metabolic syndrome, obesity and diabetes has recently been reviewed (Tang-Peronard et al., 2011). Thus, there is clearly a need for experimental evidence that elucidates the effects of environmental exposures on metabolic syndrome *per se* and not just diabetes and obesity. Specifically, studies are needed to determine the impact of chemical exposures on lipid profiles, blood pressure, altered glucose tolerance, insulin resistance and liver function in addition to weight gain.

2.10.3 Metabolic disorders in other vertebrate wildlife species

Recent efforts have been made to explore how endocrine disrupting chemicals may alter endocrine signalling and lipid homeostasis in other vertebrates, such as fish and amphbians. Tributyltin is a good example of a chemical that causes weight gain across species: It has been shown to be a potent inducer of adipogenesis in vitro and in vivo in developing toads and mice, by acting as a novel, high-affinity xenobiotic ligand for retinoic X receptor alpha (RXRα) and peroxisome proliferator-activator receptor gamma (PPARγ) (Janesick & Blumberg, 2011; Grun et al., 2006) that interacts, at least partially, with the same receptor-binding sites as other high-affinity ligands for these receptors and promotes the necessary cofactor interactions required for agonist activation. TBT binds to RXRs and activates signaling in gastropod snails, amphibians, and mammals. In all species exceopt the snails (in which masculinization occurs), TBT promotes long-term changes in adipocyte number or lipid homeostasis following developmental or chronic lifetime exposure. The fact that wildlife species are sensitive to an obesogen like TBT has led to the development of the "zebrafish obesogenic test" as a tool for screening compounds that target adiposity (Tingaud-Sequeira, Ouadah & Babin, 2011). This short-term assay, in which adipocyte droplets are visualised by fluorescence, has been used to demonstrate that zebrafish larvae treated at an environmentally relevant concentration of TBT exhibit a marked increase in adiposity. Similarly, research into the effects of long-term, dietary exposure to TBT on juvenile Chinook salmon revealed an increase in whole-body lipid content, although the analysis of associated physiological parameters revealed some inconsistencies with

metabolic syndrome (Meador et al., 2011). Probably the most environmentally-relevant studies to date, from the aquatic ecotoxicology literature, are those of Lyche et al. (2010; 2011), in which the effects of lifelong exposure to natural mixtures of POPs were analysed in zebrafish. The exposure mixtures were extracted from the livers of wild fish and contained PBDEs, PCBs and DDTs. The phenotypic effects reported included increased weight gain, advanced puberty and skewed sex ratios. Changes in the expression of genes associated with weight, homeostasis, steroid hormone functions and insulin signalling were also reported. These increased weight and gene expression changes reported suggest that environmental pollutants may affect the endocrine regulation of metabolism, possibly leading to increased weight gain and obesity in wild fish. These data are particularly pertinent given that the concentrations of POPs measured in these fish were comparable with the levels found in other species in the wild (see Chapter 3.2.1 for a review of exposure of wildlife to these chemicals). Thus, although there are still no data demonstrating that EDCs are associated with metabolic disorders in wildlife, the results of laboratory-based studies on both mammalian and non-mammalian vertebrates have revealed remarkable consistencies with the patterns reported in the human literature.

Finally, it is important to note that the discovery of "obesogens" and "metabolic disruptors" greatly expands the list of chemicals classed as EDCs, but also expands the list of receptors and hormone systems impacted by these chemicals.

2.10.4 Main messages

- Obesity, diabetes and metabolic syndrome are due to disruption of the energy storage-energy balance endocrine system and thus are potentially sensitive to endocrine disrupting chemicals.
- Obesity, diabetes and metabolic syndrome have their origins during development and are influenced by the environment during development and throughout life.
- Exposures of animal models to a variety of chemicals have been shown to result in weight gain. Because they are disrupting many components of the endocrine system involved in controlling weight gain (adipose tissue, brain, skeletal muscle, liver, pancreas and gastrointestinal (GI) tract), these chemicals constitute a new class of endocrine disruptors called "obesogens".
- Some studies report that developmental exposures of rodents to a variety of chemicals with estrogenic activity result in increased weight gain, while in adults, estrogenic activity is protective against weight gain, highlighting the importance of timing in assessment of the effects of endocrine disrupting chemicals on these diseases.
- Human epidemiological studies have shown an association between in utero exposures to several POPs, and subsequent increased weight gain in the first few years of life and that smoking during pregnancy is consistently and strongly associated with increased weight gain in infants.
- Obesity is also correlated with type 2 diabetes and chemicals that have been shown to cause obesity, in animal models, also result in altered glucose tolerance and reduced insulin resistance.
- There are strong and consistent epidemiological data linking adult exposure to dioxin and other POPs to type 2 diabetes in both cross sectional and prospective studies.
- In animal models, there are some preliminary studies showing a relationship between developmental exposures to some chemicals and glucose intolerance and insulin insensitivity later in life.
- The findings that show that developmental exposures to some environmental chemicals can lead to obesity and/or type 2 diabetes expands the likely list of chemicals and the list of hormone receptor systems, as yet not tested, that are responsive to endocrine disruptors.

2.10.5 Scientific progress since 2002

- Obesity and type 1 and type 2 diabetes have increased greatly across the globe, especially in developed countries and in children.
- Animal studies have shown that obesity, diabetes and metabolic syndrome have their origins during development and can be influenced by environmental chemical exposures, amongst other factors, during development and throughout life.
- There are human epidemiological data linking adult exposure to POPs and diabetes and data showing developmental exposures to a variety of chemicals can lead to weight gain in children.
- A new class of endocrine disrupting chemicals that cause obesity, called "obesogens", has arisen.

2.10.6 Strength of evidence

There is sufficient evidence to show that over the last three decades, the global prevalence of obesity, diabetes and metabolic syndrome have increased based on comprehensive global surveys. There is also sufficient evidence that these diseases have an endocrine origin and thus are susceptible to disruption by environmental chemicals with endocrine function. The hypothesis that environmental chemical exposures are playing a significant role in the etiology of obesity is really less than a decade old and there is currently a lack of human literature for many of the chemicals shown to cause effects in animal studies. In addition, many of the animal studies are descriptive and have not shown specific endocrine mechanisms of action. The "obesogen" hypothesis, while now encompassing close to 20 environmental chemicals and classes, is still considered

an emerging hypothesis. The data on tributyltin and bisphenol A and obesity are sufficient in animal studies but there are no human studies. The data linking environmental chemical exposures to diabetes or metabolic syndrome are insufficent, due to lack of both animal and human data. The strongest data are for POPs and type 2 diabetes, as the human data are consistent, but there is a lack of mechanistic insight from animal studies. Thus the potential for the "obesogen" hypothesis as a mechanistic explanation for various metabolic diseases is great. However, the field needs more data in both animal and human studies, including stronger linkages to endocrine mechanisms of action.

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2.11 Endocrine disruptors and immune function, immune diseases, and disorders in humans and wildlife

2.11.1 Overview of immune diseases in humans and wildlife and evidence for endocrine disruption

Alterations in the immune system such as immune modulation, hypersensitivity, and autoimmunity can lead to a decreased quality of life. Laboratory studies with animals show that many chemicals introduced into the environment have the potential to disturb the immune system of wildlife and humans. The consequences of such interference on the developing immune system are not, however, well understood. Nonetheless, there are certain situations where human and wildlife populations are experiencing immune alterations that are consistent with those produced by synthetic chemicals identified as immunotoxic in studies with laboratory animals:

- **Human allergic diseases constitute the most common causes of chronic illness** in developed countries and incidences are rising in developing countries.
- **Human autoimmune diseases are also rising**. To date, more than 80 systemic and organ-specific autoimmune diseases have been defined, and their cumulative burden is substantial, both medically and financially. In developed countries around the world, 5 to 7% of the population is affected and rates are rising, making these diseases a ubiquitous global phenomenon that is predicted to further increase in the coming decades (an expert panel reported at the "Global State of Autoimmunity" briefing for international health ministers hosted by the United Nations' NGO Health Committee and the American Autoimmune Related Disease Association (AAARD) on 25 September 2010).
- **Rising prevalence rates of autoimmune disease in humans are most evident in type 1 diabetes.** Data from Finland, tracked by that country's national health system, show type 1 diabetes rates more than doubled from 31 cases per 100 000 people in 1980 to 64 cases per 100 000 in 2005 (Harjutsalo, Sjoberg &Tuomilehto, 2008) Increases were also detected in 17 other European countries, at an average annual rise of 3.9% from 1989 to 2003 (Patterson et al., 2009). The authors of that study predicted the number of new cases in children younger than 5 years in Europe will double by 2020 compared with 2005, while the number of cases among those under age 15 will rise by 70%.
- **High rates of Chytridomycosis and Rana virus in amphibian populations have raised concern about the sustainability of affected populations**, especially those that are threatened or endangered.
- **Fibropapillomas have become an important emerging disease of sea turtles** since the early 1980s.
- **Marine mammals worldwide have been affected by emerging disease during recent years.** At least 20 species of cetaceans (whales, dolphins and porpoises) and 15 species of pinnepids (seals, sea lions and walruses) have been affected by more than 30 different emerging and re-emerging disease conditions.
- **Disseminated neoplasia (DN) emerged during the 1980s and was first described in New England (USA) in soft-shelled clams.** DN has been compared to vertebrate leukemia as a disease process.

The causes of these disease trends are unknown. It is clear that autoimmunity is a multifactorial process in which genetics, immunological, environmental and hormonal factors act in concert, together representing what was termed years ago the 'mosaic of autoimmunity'. Genetics plays a key role in susceptibility to autoimmune disease (among identical twins, for example, if one has an autoimmune disease, there is a 30 % chance the other twin will also develop one, though not always the same disease). Whilst genetics may "load the gun", the environment appears to pull the trigger, explaining up to 70% of the risk of some diseases (Ramos & Olden, 2008). For example, lupus (an autoimmune disease commonly affecting the joints, skin, kidneys, blood cells, heart, and lungs) is four times more likely to strike people of African descent who live in London than those who live in sub-Saharan Africa. Globally, a similar trend applies to other autoimmune diseases, since autoimmune disease remains uncommon in developing countries, while increasing in developed nations. Human evidence for other environment-disease links is more tenuous, however, in part because of inherent limitations in environmental epidemiology. By contrast, effects of chemicals on immune function in free-ranging marine mammals have been inferred from a number of studies (Beckmen et al., 2003; Lahvis et al., 1995; Beineke et al., 2005), the most comprehensive of which are studies on the immunotoxic effects of contaminants in harbour seals (*Phoca vitulina*) (De Swart et al., 1994; 1995a; 1995b; 1996a; 1996b; Ross et al., 1995; 1996).

Many immune disorders are deeply rooted in the endocrine system and, therefore, inappropriate activation or inactivation of select endocrine pathways may aberrantly disturb the balance of the immune response. This is due to the fact that the immune and the endocrine systems are intricately connected, ensuring that the body can simultaneously handle infections, stress, the immune response, and hormonal signalling. Cytokines, small cell signalling molecules, traditionally thought to have an immunomodulatory role, may also be crucial regulators of autocrine-paracrine effects of hormones and may send and receive signals (Khardori, Adamski & Khardori, 2007). EDCs have been linked with disorders of metabolism, energy balance, thyroid function and reproduction, as well as an increased risk of endocrine cancers (Walker & Gore, 2011).

This chapter discusses the current data linking EDC exposure to immune disorders and stresses the need for further research to deepen our understanding of the effect of EDCs on the immune system.

2.11.2 Evidence for endocrine disruptor causation of endocrine-immune diseases and disorders in humans and in rodent models

2.11.2.1 Interaction between the immune and endocrine systems

The interaction between the immune and endocrine systems has been well documented, as have the immunomodulatory effects of various hormones (Elenkov & Chrousos, 2002), particularly glucocorticoids secreted by the adrenal cortex (Oberbeck, 2004; see section 2.8). Other compounds and neurotransmitters known to affect immune function include the adrenal androgen dehydroepiandrosterone (DHEA; section 2.8), the catecholamines adrenalin, noradrenalin, and dopamine, and the pituitary hormone prolactin (Oberbeck, 2004). Catecholamines, prolactin, and DHEA levels become elevated following major surgery and during systemic inflammation (Oberbeck, 2004), and prolactin may both stimulate the immune response and reduce the release of glucocorticoids following a stress (Davis, 1998). Growth hormones (GH), insulin-like growth factor-I (IGF-I), glucocorticoids, and thyroid hormones also have established interactions with the immune system; stress, nutrition, and environment may mediate their function (Davis, 1998). Growth hormone has been implicated as being involved in leukemia and lymphoma (Hooghe, et al., 1998), and it may stimulate proliferation of beta cell-derived tumours (Baglia, Cruz & Shaw, 1992). Glucocorticoids are immunomodulatory (specifically, immunosuppressive), and are frequently prescribed following organ transplantation, for serious allergic reactions or for autoimmune incidents. Together with other select chemotherapies, glucocorticoids affect both the innate (immediate) and the adaptive (long-lasting) arms of the immune response (Flammer & Rogatsky, 2011). Thyroid-stimulating hormone (TSH) is produced by a variety of immune cells, including B and T lymphocytes, splenic dendritic cells, hematopoietic cells in the bone marrow, and other lymphocytes (Klein, 2006).

The hypothalamic-pituitary-adrenal (HPA) axis is known to regulate a variety of immune functions (Marx et al., 1998; see also section 2.8). Activation of this axis generates a stress response, which consequently affects the immune response. Interactions between the HPA axis and the immune system may result from a cytokine-regulated feedback mechanism. The adrenal gland (the major effector organ of the HPA axis) also produces a variety of cytokines, implicating the adrenals as crucial players in the immuno-endocrine intersection (Marx et al., 1998). Cytokines may also be produced by activated immune cells, and these cytokines can subsequently direct hormone secretion from the HPA axis or the hypothalamic-pituitary-gonadal (HPG) axis. In the other direction, hormones (both adrenal and sex steroids) may direct the production of cytokines by immune cells (Marx et al., 1998). Macrophages in the ovary can also produce cytokines that affect the follicle and corpus luteum and, as a result, may affect ovarian steroidogenesis. In turn, these ovarian steroids may also affect the cytokines produced by the macrophages. Estrogen receptors have been detected in immune cells such as dendritic cells, macrophages, and B cells (Nalbandian & Kovats, 2005), suggesting that estrogens (or xenoestrogens) may act on these cells (Chryssikopoulos, 1997). In general, testosterone is viewed as largely anti-inflammatory, whereas estrogen is thought to be more pro-inflammatory (Klein, 2004; McClelland & Smith, 2011). Testosterone also suppresses the immune system and increases susceptibility to infection (Schuurs & Verheul, 1990). These data are in accord with the fact that autoimmune and inflammatory disorders are found in a disproportionately higher incidence in females versus males (Whitacre, 2001). Finally, mucosal immunity (which protects an organism's various mucous membranes from invasion) and immunosenescence are thought to be under the control of steroid hormones via their effects on cytokine production (Daynes et al., 1995). Together, these data support a role for steroid and sex hormones in immunity and it is likely that disruption of normal steroid signalling by exogenous chemicals may affect immune function.

2.11.2.2 Mechanisms via which EDCs might influence the immune system

Nuclear hormone receptors and inflammation

Although nuclear receptors are best known for their direct effects of activating transcription of target genes by binding to hormone response elements in the DNA, nuclear receptors are also well known for their signaling crosstalk with the immune system, particularly through NF-κB and AP-1 (De Bosscher, Vanden Berghe & Haegeman, 2003; Valledor & Ricote, 2004; Pascual & Glass, 2006). Nuclear Factor Kappa B (NF-κB) is a family of transcription factors that controls the transcription of DNA and is activated in response to stress, bacterial or viral antigens or an inflammatory stimulus. Upon activation of either the T- or B-cell receptor, NF-κB family members become activated through distinct signalling components and play a key role in regulating inflammation and the immune response to infection (Baeuerle & Baltimore, 1996). In a resting cell, NF-κB proteins are in an inactive state in the cytoplasm, but upon an inflammatory signal, the proteins are released from inhibition and migrate to the nucleus to activate transcription of a variety of target genes (Baeuerle & Baltimore, 1996) that control cell proliferation and cell survival to protect the cell from conditions that would otherwise cause it to die via apoptosis. As such, many different types of human tumours have misregulated NF-κB.

NF-κB is known to crosstalk with a variety of nuclear receptors, among them, the glucocorticoid receptor (GR) that mediates the actions of glucocorticoids, a subset of steroid hormones produced by the adrenal cortex after the HPA axis is activated by inflammation (Elenkov & Chrousos, 2006; Sternberg, 2006; Bowers et al., 2008). Glucocorticoids may inhibit NF-κB through a variety of mechanisms and this process is likely cell type-specific (Nissen & Yamamoto, 2000; Liberman et al., 2007; Bowers et al., 2008) and mediated by the glucocorticoid receptor (Heck et al., 1994; Liden et al., 1997; Tao, Williams-Skipp & Scheinman, 2001). Estrogen receptor (ER) function has also been linked with constitutively active NF-κB, and this may have profound consequences for aggressive hormone-resistant cancers (De Bosscher, Vanden Berghe & Haegeman, 2006). It has also been shown that binding of NF-κB to target genes can be blocked by estrogens in vitro (Stein & Yang, 1995) and that activation of the progesterone receptor (PR) can reduce NF-κB-driven gene expression (Kalkhoven et al., 1996). Reciprocal negative cross-talk has also been documented between NF-κB and the androgen receptor (AR) (Palvimo et al., 1996). All of these pathways offer possible mechanisms for EDCs to impact immune function through receptor-mediated crosstalk with NF-κB. Additionally, "classical" ARs can activate the Map Kinase (MAPK) pathway through the epidermal growth factor (EGF) receptor (Cheng Watkins & Walker, 2007), and androgens influence T cell development and cytokine development (Bebo et al., 1999). This identifies a role for AR in inflammation and provides an additional immune signalling pathway through which EDCs may act.

Several groups have demonstrated that activation of the Steroid and Xenobiotic Receptor (SXR; also known as PXR and NR1I2) by commonly used pharmaceuticals inhibits the activity of NF-κB (Gu et al., 2006; Zhou et al., 2006). NF-κB target genes are up regulated in cells and tissues lacking SXR and inhibition of NF-κB function boosts SXR activity. Activation of NF-κB inhibits SXR activity while activation of SXR inhibits NF-κB activity (Gu et al., 2006; Zhou et al., 2006). The SXR/NF-κB axis provides a potential molecular explanation for suppression of cytochrome P450 genes by inflammation and infection and clarifies the immunosuppressant role of pharmaceuticals that activate SXR. Consequently, many xenobiotics or EDCs that activate or inactivate SXR could be responsible for regulating inflammation through NF-κB.

Lastly, it has been shown that peroxisome proliferator-activated receptor gamma (PPARγ) mediates repression of NF-κB (Delerive et al., 1999) and that PPARα activation suppresses inflammation (Yu, et al., 1995; Devchand et al., 1996; Lehmann et al., 1997). Since EDCs such as phthalate monoester plasticizers are known to activate both rodent and human PPARα and PPARγ (Hurst & Waxman, 2003), biologically plausible that environmental chemicals acting through PPARα may have strong negative impacts on human immune function. Additionally, other receptors such as the Liver X Receptors may cross-talk with NF-κB (Joseph et al., 2003; Wu et al., 2009). Liver X Receptors (LXRs) are known regulators of lipid-inducible gene expression that negatively regulate inflammatory gene expression in macrophages (cells known to have important roles in lipid metabolism and in inflammation). Stimulation of macrophages with lipopolysaccharide demonstrated that NF-κB target genes such as interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) were inhibited by LXR ligands in vitro. LXR agonists reduced inflammation in both the skin and aorta of in vivo models, securing a role for LXR as a key mediator of lipid metabolism and inflammation (Joseph et al., 2003). It was recently shown that EDCs such as bisphenol A affect the expression of LXR (Marmugi et al., 2011) and that LXR affects estrogen metabolism through expression of estrogen sulfotransferase, a transcriptional target of LXR (Gong et al., 2007). Therefore, it is plausible that EDCs, particularly xenestrogens, may exert at least a fraction of their effects via LXR. Further research is needed to fully understand the role of LXR in endocrine diseases that could be partially or wholly caused by exposure to endocrine disrupting chemicals.

2.11.2.3 Endocrine-immune diseases and disorders in humans

Allergies

Allergies are becoming increasingly common, particularly in younger individuals. Data suggest that the incidence of childhood allergy may be connected to environmental aspects such as stress, but a growing amount of work indicates that disturbances of the fashion in which the HPA axis responds to stress may be to blame. It is possible that an over reactive or under reactive HPA axis during the initiation and continuation of childhood allergy may contribute to the allergic response (Buske-Kirschbaum, 2009). Since select nuclear receptors (such as ER) interact with CRH-binding protein (CRH-BP), which can activate the HPA axis by binding and inhibiting CRH, it is plausible that EDCs could ultimately lead to an under reactive or over reactive HPA axis (van de Stolpe et al., 2004).

Endometriosis

Endometriosis is a common gynaecological disorder that is characterized by the presence of endometrial cells or tissue outside of the uterine cavity, usually in the ovaries. It is commonly thought that retrograde menstruation is to blame for endometriosis (Sampson's theory) (Seli & Arici, 2003; Jensen & Coddington, 2010), but because the rate of endometriosis is far lower than the incidence of retrograde menstruation (over 75% of women), it is likely that other factors contribute to susceptibility. The disease is linked with changes in both cell-mediated and humoral immunity; dysfunctional natural killer cells may not properly remove refluxed menstruation debris, and this may lead to endometriotic implants (Seli & Arici, 2003). Additionally, larger numbers of immune cells are found in the peritoneal cavity fluid of women with the disease, but these cells appear to be positively correlated with disease progression. Cytokines and growth factors secreted by

macrophages (which usually help in clearing up tissue debris) may foster the growth of endometrial cells and it is possible that they contribute to endometriosis-associated infertility and pelvic pain (Seli & Arici, 2003). Danazol (a derivative of the synthetic steroid ethisterone, a modified testosterone) and gonadotrophin-releasing hormone (GnRH) activators are often used in clinics for treatment of endometriosis; they dampen the cellular and humoral immune responses (in addition to their effects on endometrial cells outside the uterine cavity) (Seli & Arici, 2003). It is likely that the immunosuppressant activity of these compounds contributes greatly to their therapeutic value, implicating the immune system in the disease. It has been suggested (McLachlan, Simpson & Martin, 2006) that endometriosis may be at least partially attributed to exposure to EDCs (also discussed in section 2.2).

Autoimmune thyroid disease

Autoimmune thyroid diseases (AITD) are the most frequently occurring organ-specific autoimmune disease; approximately 5% of the population is thought to be affected (Klecha et al., 2008). Autoimmune thyroid disease is caused by abnormal interactions between atypical thyrocytes (cells of the thyroid gland), aberrant antigen-presenting immune cells, and abnormal T lymphocytes, with the end phenotype being an immune reaction against "self" that attacks antigens in the thyroid (Klecha et al., 2008). It is possible that environmental or hormonal influences may affect the etiology of this disease, perhaps by changing the normal immune-endocrine interaction. Alterations of the healthy immune-endocrine axis may tip the delicate balance between the two types of immune response, which ultimately leads to either Hashimoto thyroiditis (hypothyroidism and destruction of thyrocytes) or Graves disease (an immune response against the TSH receptor, leading to hyperthyroidism) (Klecha et al., 2008). Stress, environment, immune function, and thyroid hormones may all be affected by EDCs (see also section 2.5) and, consequently, influence the development of autoimmune thyroid disease (Diamanti-Kandarakis et al., 2009; Zoeller, 2010).

Bone disorders

The immune system plays an important role in osteoporosis, which often arises from estrogen deficiency and secondary hyperparathyroidism (excessive production of parathyroid hormone (PTH) by the parathyroid glands situated at the back of the thyroid gland). Current research suggests that the remodelling of bone is a very tightly controlled process that is easily perturbed by small fluctuations in pro-inflammatory and inhibitory cytokines, NF-κB, together with hormones and their corresponding receptors (Clowes, Riggs & Khosla, 2005; see also section 2.9). An imbalance in this interplay, due to infection or inflammation, could tip the bone creation/bone destruction scale in favour of bone loss, which subsequently increases the risk of fracture. Furthermore, age-related fluctuations in the immune and endocrine systems add to the risk for decreased bone density. It is possible that exposure to EDCs may influence the development of osteopenia and osteoporosis.

2.11.2.4 Evidence that exposure to EDCs causes immune system disorders in humans

EDCs and endometriosis

There are data that correlate phthalate plasticizer (Chapter 3.1.1.3) levels in plasma and endometriosis. Cobellis et al. found high plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis (Cobellis et al., 2003) and the concentration of phthalate esters was also linked with endometriosis in a study of Indian women (Reddy et al., 2006). Human exposure to phthalates is extensive and is reviewed in Chapter 3.2.2 PCBs (Chapter 3.1.1.1) CB-138, CB-153, and CB-180 (Gerhard & Runnebaum, 1992), as well as dioxins (Koninckx et al., 1994), have also been loosely associated with the disease. Mayani et al. (1997) reported that dioxin exposure (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) was positively correlated with endometriosis, although it is not possible to conclude from these data that TCDD was the sole cause of the disease. Data collected from accidental dioxin exposure after an industrial accident in Seveso, Italy, revealed an increased risk of endometriosis (Eskenazi et al., 2000), but this study was limited by difficulties in disease classification and statistical power. Dioxin-like compounds have been found in elevated levels in the blood of women with endometriosis (Heilier et al., 2005), as have PCBs (Porpora et al., 2006) in a study of women who had not had children (thereby controlling for fluctuation of chemicals during breastfeeding; see Chapter 3.2.2.6 for exposure through mothers' milk). Although evidence is mounting that correlates EDCs found in the bloodstream of women with endometriosis, data that suggest a mechanism, or that establish causal links, are scant. Dioxins activate the aryl hydrocarbon receptor (AhR) (Yoshioka, Peterson & Tohyama, 2011) and dioxin bound to AhR interacts with estradiol, leading to cell growth in the mouse uterus (Ohtake et al., 2003). More recently, it was shown that AhR mediates non-genomic actions of AhR-ligands and promotes the degradation of ERα and AR (Ohtake et al., 2011). Taken together, these findings may provide insight for future epidemiological studies, and indicate that additional work on EDC exposure and endometriosis is needed.

Asthma

A variety of EDCs, including estrogenic compounds, are known developmental immunotoxicants (Fenaux, Gogal & Ahmed, 2004; Guo et al., 2006). The developing and neonatal immune response is easily affected by EDCs, and disruption during critical windows of development may have detrimental long-term consequences. Developmental immunotoxicity (DIT) caused by EDC exposure may be one early-life immune insult that could cause lifelong effects on immunity and the overall health of exposed individuals. Evidence from studies with mice suggests that exposure to certain EDCs, such as diethylstilbestrol (Fenaux, Gogal & Ahmed, 2004) and genistein (Guo et al., 2006), may lead to postnatal immune disorders such as asthma. Epidemiological studies further suggest that exposure to

chemicals may be involved in the etiology of childhood asthma or cancers such as childhood leukemia (Dietert, 2009). The incidence of childhood allergic disease (including asthma) has increased since the mid-1900s, and this increase is thought to be linked with changes in the environment. Predisposition toward allergic disease is one of many adverse outcomes that could be caused by developmental immunotoxicity. Environmental estrogens and other EDCs have been identified as contributors to developmental immunotoxicity-related immune disorders (Dietert & Zelikoff, 2008).

Phthalates and asthma

Phthalates are used in soft polyvinylchloride (PVC) material and are present in many household materials and personal care products. The most common phthalate indoors is DEHP, which can be found in cosmetics, toys, construction material, and cleaning solutions (Schettler, 2006). An epidemiological study examining the association between PVC products in the home and the incidence of airway symptoms determined that the presence of PVC materials increased the risk for bronchial obstruction in young children (Oie et al., 1999; Chalubinski & Kowalski, 2006). Additional studies from Sweden, Russia and Finland supported this finding and showed that exposure to PVC flooring and/or PVC wall covering material was correlated with airway symptoms in children (Jaakkola, Verkasalo & Jaakkola, 2000; Bornehag et al., 2005). Moreover, two case-control prevalence studies from Sweden and Bulgaria describe an association between the concentration of DEHP in indoor dust and asthma and wheezing in children (Bornehag et al., 2004; Kolarik et al., 2008). Taken together, these studies are in accord with published animal studies (Bornehag & Nanberg, 2010).

Incubating peripheral blood mononuclear cells from allergic individuals with mono-n-butyl phthalate (MnBP) elevated the production of interleukin-4 (IL-4; Glue et al., 2002). Moreover, mono-2-ethylhexyl phthalate (MEHP) was shown to potentiate the allergic reaction to ovalbumin (OVA; a commonly-used allergen) exposure in a mouse inhalation model (Hansen et al., 2007). Increased levels of OVA-specific immunoglobulins in the serum, as well as an increase in eosinophilic (acid loving) white blood cells, were observed. When cells harvested from the draining lymph nodes were cultured, cytokine expression was indicative of a activation of a helper cell (Th2) immune pathway (Hansen et al., 2007); the helper cells activate and direct other immune cells.

Triclosan and asthma

Triclosan, an antimicrobial agent found in many household soaps, toothpastes, and disinfectants, was investigated for links to allergies and hay fever in the United States. Triclosan levels in children under 18 years of age were tightly correlated with development of these diseases in data taken from the 2003-2006 USA NHANES database (Clayton et al., 2011). These findings suggest that further work on triclosan and its correlation with immune and respiratory diseases is needed (for more information on exposure pathways for triclosan see Chapter 3.1.1.5).

Autoimmune thyroid disease

Many have hypothesized that diet during early life may influence immune disorders that develop later in life (Fort et al., 1990). Since thyroid dysfunction is one of the most common autoimmune diseases early in life, it is possible that EDCs play an important role in the development of these diseases (see also section 2.5). For example, soy-based milk formulas given to children are linked with a higher incidence of autoimmune thyroid disease as compared to their breast-fed siblings (Fort et al., 1990). On the other hand, environmental chemicals may also be linked with autoimmune thyroid disease. While iodine is the most well-known environmental factor associated with this disease, limited epidemiological data also show that methylcholanthrene, furan, polybrominated biphenyls, PCBs (Guarneri & Benvenga, 2007), and polyaromatic hydrocarbons (Burek and Talor, 2009) may be associated with autoimmune thyroid disease. PCBs were associated with thyroid disease in data collected from a heavily polluted area in Slovakia; of particular epidemiological note in this study was that autoimmune antibodies were found in the offspring of exposed individuals (Langer et al., 2008) even though their levels of organochlorines were far lower than those of their parents, suggesting that the effect of EDCs on autoimmune thyroiditis may be multigenerational. It is unclear whether the effect is due to direct PCB transfer in utero or in breast milk, or whether this effect is due to an epigenetic phenomenon (for a review of human exposure to POPs see Chapter 3.2.2 and for a description of epigenetics see Chapter 1.3.6).

EDCs, dendritic cells, and cytokines

Dendritic cells are antigen-presenting cells that act as messengers between the innate and adaptive immune systems. They are present in tissues in contact with the external environment, such as the skin and the inner lining of the nose, lungs, stomach and intestines. Dendritic cells can also be found in an immature state in the blood. Once activated, they migrate to the lymph nodes where they interact with T cells and B cells to initiate and shape the adaptive immune response. At certain development stages they grow branched projections, the dendrites that give the cell its name.

Cultured human circulating dendritic cells (mDCs) treated with two common EDCs, nonylphenol (NP) or 4-octylphenol (4-OP), demonstrated changes in anti-inflammatory cytokine production that was partially reversible by an estrogen receptor antagonist. Overall, the findings suggested that NP and 4-OP affected the immune response of dendritic cells through the estrogen receptor (ER) (Hung et al., 2010). Some EDCs also boost STAT3 mediated signaling through ER (Sekine et al., 2004). STAT3 plays a key role in many cellular processes such as cell growth and apoptosis and is mostly activated by interleukin-6 (IL-6), also a target for NF-kB (Kishimoto, Taga & Akira, 1994). The strong STAT3 activation by EDCs through ER can be reversed by the anti-estrogen tamoxifen (Sekine et al., 2004). EDCs can also activate MAP kinase (MAPK) signalling, which suggests that EDCs can interfere with

normal endocrine and immune homeostasis by influencing the cytokine signalling pathways as well as through direct binding to estrogen receptors (Sekine et al., 2004).

Organotins and immune dysfunction

Triphenyltin (TPT) is a well-known EDC, but little is known about its effects in the immune system. TPT increased superoxide production by approximately 45% and increased expression of crucial neutrophil proteins that endow the cell with its capacity to migrate towards and eliminate microbial pathogens, by about 90% (Watanabe et al., 2003). Other EDCs tested (e.g. parathion, vinclozolin, and bisphenol A) did not have these effects. Microarray data also supported the conclusion that TPT elevated the expression of crucial neutrophil proteins, thus enhancing the neutrophilic maturation of leukocytes. Dibutyltin (DBT) has been found to inhibit binding of ligands to the glucocorticoid receptor (GR) and, subsequently, its activation (Gumy et al., 2008). DBT also inhibited the action of the enzyme 11-β hydroxysteroid dehydrogenase, thereby increasing circulating glucocorticoid levels (Odermatt & Gumy, 2008). DBT inhibited glucocorticoid-mediated inhibition of inflammatory cytokines in stimulated macrophages, suggesting that DBT could have potent effects on immune processes (Gumy et al., 2008). TBT and TPT are also strong inhibitors of human natural killer (NK) cell function as measured by tumour cell lyses (Whalen, Loganathan & Kannan, 1999; Whalen, Hariharan & Loganathan, 2000; Wilson et al., 2004; Gomez, et al., 2007). In summary, these findings imply that organotins may play a key role in immune disruption, can inappropriately stimulate cytokine signaling, and might reduce the ability of natural killer cells to effectively eliminate their targets. Furthermore, exposure to these compounds could be associated with an increased risk of cancer, as the immune system may not be able to lyse tumour cells.

Childhood lymphomas and leukemias

It has been suggested that occupational exposure to hydrocarbons in parents is associated with an increased risk of childhood leukemia (Fabia & Thuy, 1974; Shu et al., 1999). A single study from the Children's Cancer Group, that examined self-reported occupational exposure to a variety of hydrocarbons, revealed that exposure to solvents and paints or thinners during a preconception window and during pregnancy and exposure to plastics within the postnatal window were correlated with an elevated childhood risk of acute lymphocytic leukemia (Shu et al., 1999). Additionally, exposure to plastics pre-conception was also linked to acute lymphocytic leukemia (Shu et al., 1999).

Nuclear hormone receptors and lymphomas in rodent models

The steroid and xenobiotic receptor (SXR) is a broad-specificity nuclear hormone receptor that is highly expressed in the liver and intestine, where its primary function is to regulate drug and xenobiotic metabolism (Zhou, Verma & Blumberg, 2009). Mice lacking SXR demonstrate aberrantly high NF-κB activity and over expression of NF-κB target genes and ultimately develop B cell lymphoma in an age-dependent manner. SXR knockout mice develop lymphocytes with cell surface and molecular characteristics of either chronic lymphocytic leukemia or non-Hodgkin lymphoma (Casey et al., 2011), providing a link between metabolism of xenobiotic compounds and the initiation of lymphoma (Casey et al., 2011). It is plausible that chemical antagonists of SXR would be associated with the development of lymphoma, and inhibition of the xenobiotic response may be a key step in the development of certain lymphoid tumours (Casey et al., 2011).

In addition to SXR, rodent studies also show that loss-of-function in several other nuclear receptors has been linked with proliferation of lymphoid cells. Loss of the nuclear receptor LXR resulted in enhanced proliferation of T cells, connecting sterol metabolism to the acquired immune response (Bensinger et al., 2008). B cell lymphomas also developed in aged steroid receptor coactivator-3 null mice (Coste et al., 2006) and retinoid-related orphan receptor ROR gamma knockout animals displayed lymphocyte accumulations in the spleen (Zhang, Guo & He et al., 2003) and T cell lymphoma (Ueda et al., 2002). Loss-of-function in the nuclear receptors Nr4a3 and Nr4a1 also leads to acute myeloid leukemia (Mullican et al., 2007). These data support a role for nuclear hormone receptors in the regulation of lymphocyte proliferation and in lymphomas and leukemias.

Links between PCBs, POPs and hematopoietic disorders and malignancies

Xenobiotic chemicals and environmental pollutants, particularly PCBs, have long been associated with increased risk of non-Hodgkin lymphoma (Bertrand et al., 2010 ; Maifredi et al. 2011; Rothman et al., 1997; De Roos et al., 2005; Engel et al., 2007; Spinelli et al., 2007). PCBs were commonly produced in North America for over half a century, but now banned worldwide. PCBs remain persistent environmental contaminants (see Chapter 3.2 for a review of exposure to PCBs in humans and wildlife). Chang et al. (1981) found that children exposed to PCBs in utero displayed decreased immunoglobulin levels, and had fewer total T cells. Moreover, a study of Flemish 17 and 18 year olds indicated that elevated serum PCBs correlated with decreases in immune cells such as eosinophils and natural killer cells, decreases in serum immunoglobulins IgE and IgG, and an increase in serum immunoglobulin IgA (Van Den Heuvel et al., 2002).

Persistent organic pollutants (POPs), particularly organochlorine pesticides, have been associated with other immune-based disorders. For example, they were positively correlated with periodontal disease, which is likely due to immunomodulation. Moreover, neutrophil counts in peridontal disease patients were negatively correlated with organochlorine levels (Lee, Jacobs & Kocher, 2008).

Pesticides and childhood leukemias

Several studies have connected pesticide exposure and the development of leukemia in children (Birnbaum & Fenton, 2003). Unfortunately, many of these studies do not have

detailed exposure information, only studied small numbers, and are limited by a recall bias of the parents (Belson, Kingsley & Holmes, 2007; Infante-Rivard & Weichenthal, 2007). In one study, the California Department of Health Services found that in utero exposure to metham sodium (odds ratio of 2.05; 95% confidence interval=1.01-4.17) and dicofol (odds ratio of 1.83; CI=1.05-3.22) were associated with a higher incidence of early-childhood leukemia; dicofol has been found to have estrogenic activity (Guillette et al., 1994; Okubo et al., 2004) and may function as an EDC. More work is needed to uncover the role of endocrine disrupting pesticides and childhood leukemias and lymphomas. In summary, it is feasible that selected pollutants may have strong effects on immune alteration, immune disorders, and hematopoietic cancers.

EDCs and prostate inflammation in rodent models

Gilleran et al. (2003) found that exposure to estrogens in the embryonic rat ultimately leads to disrupted development of the prostate gland. In the resulting adult offspring, the prostate gland developed epithelial dysplasia and chronic inflammation comprised of T lymphocytes and macrophages, a phenotype seen in chronic prostatitis (Gilleran et al., 2003). Furthermore, males that received estradiol after birth displayed larger spleens and thymuses, and smaller prostates and testes, with prostate inflammation and immune infiltration that was both prolactin dependent and independent. Since benign prostatic hyperplasia (BPH) often precedes prostate cancer in older male humans, these findings are of particular interest for the study of the relationship between exposure to xenoestrogens and the ultimate development of prostate cancer.

Bisphenol A and inflammation

Bisphenol A is used in food packaging and is also found in dental sealants, a variety of plastics, resins, and cosmetics (Chapter 3.1.1.5). In comparison to phthalates, there is less literature demonstrating the associations between human bisphenol A exposure and development of asthma or allergy, but available studies suggest that bisphenol A exposure is pro-inflammatory. Ex vivo exposure to bisphenol A resulted in elevated interleukin-4 (IL-4) in lymph node helper (Th2) cells in mice (Tian, Takamoto & Sugane, 2003). Prenatal (Yan, Takamoto & Sugane, 2008) and early postnatal (Sawai, Anderson, & Walser-Kuntz, 2003) exposure of mice generated a greater Th2 response (Kwak et al., 2009). Perinatal bisphenol A exposure enhanced allergic sensitization and bronchial inflammation and responsiveness in vivo (Midoro-Horiuti et al., 2010). Moreover, human monocyte-derived dendritic cells exposed to bisphenol A in vitro produce cytokine signals that favour development of the Th2-dominated responses in allergic reactions (Guo et al., 2010). Lastly, BPA enhanced the production of immunoglobulin M (IgM) from certain types of lymphocytes (which often produce autoantibodies) (Yurino et al., 2004), further implicating bisphenol A in inflammatory diseases. Taken together, these data indicate that exposure to bisphenol A could be a factor in the development of inflammatory and autoimmune diseases.

Atrazine and immune function

Atrazine, as well as its main metabolite, desethylatrazine, are the most prevalent groundwater contaminants in agricultural, undeveloped, and urban-use areas in the United States (Barbash et al. 2001; see Chapter 3.1.1.6). When human peripheral blood mononuclear cells from healthy donors were treated with atrazine in vitro, production of interferon gamma (a type 1 cytokine), interleukin-5 (a type 2 cytokine) and TNFα (an inflammatory cytokine) were reduced to a level comparable to the positive control - the known immunosuppressant dexamethasone (Hooghe Devos & Hooghe-Peters, 2000). When purified natural killer cells were exposed to 10 μM atrazine, a significant decrease in lytic function was observed, suggesting that atrazine has potent immunomodulatory effects on both T cells and natural killer cells (Whalen et al., 2003). It is thought that atrazine exposure blocks the ability of natural killer cells to lyse their target cells by inhibiting lytic granule release without affecting the ability of these cells to form interactions with target cells (Rowe, Brundage & Barnett, 2007).

In a rodent model, exposure during the prenatal period and during lactation resulted in a sex- and age-dependant depression of immune function in the adult offspring (Rowe, Brundage & Barnett, 2007). Atrazine treatment was also shown to reduce the number of naïve T helper and T cytotoxic cells in the spleen of treated animals, while it increased the percentage of activated cytotoxic T cells, and inhibited dendritic cell maturation in the spleen (Filipov et al., 2005). In a separate study using a Balb/c mouse model, prenatal/lactational exposure to atrazine altered adult immune function and led to an increase in both T cell proliferation and cytolytic activity in male offspring, and the humoral immune response was also significantly increased (Rowe et al., 2006). Another study showed that atrazine directly targets maturation of dendritic cells and that EDCs (such as atrazine) that remove MHC-I molecules on the surface of dendritic cells are likely to have a potent role in immune evasion (Pinchuk, Lee & Filipor, 2007). Together, these data indicate that atrazine has convincing effects on the immune system, and further investigation is warranted.

Arsenic

Millions of people worldwide are exposed to arsenic, a toxicant that is associated with increased risk for a variety of cancers and cardiovascular disease. Arsenic treatment of an in vitro mast cell model inhibited antigen-stimulated degranulation at environmentally-relevant, non-toxic concentrations, suggesting that arsenic may reduce overactive immune responses, and, additionally, may inhibit normal immune responses against immune insults such as parasitic disease (Hutchinson et al., 2011). Furthermore, these data imply that different EDCs work in different ways on mast cell degranulation and may have unique responses on the immune response in asthma, and in allergies.

2.11.3 Evidence for endocrine disruptor causation of endocrine-immune diseases and disorders in wildlife

The potential for widespread effects of endocrine disrupting chemicals on the immune systems of wildlife is significant, but less understood than their potential for disruption of reproductive health and development. As EDCs are typically found as complex mixtures in a changing environment, wherein additive, antagonistic or synergistic interactions may be expected, the science of immunotoxicology must overcome a number of challenges including:

- chronic exposures to EDCs in diet and/or environment.
- exposures to complex mixtures where determining causation associated with any given contaminant or class of contaminants is problematic.
- constantly changing profiles of contaminants as new chemicals emerge and legacy chemicals decline.
- confounding factors such as age, sex, and condition that can affect EDC concentration as well as the health of the individual.
- the interacting effects of multiple stressors in addition to EDCs, such as climate change, habitat alteration, invasive species, and/or eutrophication.
- legal, technical and ethical constraints to working with wildlife, notably those listed under endangered species legislation.

Dealing with these challenges will require new concepts, tools, and approaches.

The evidence for immunotoxic effects resulting from EDC exposure in wildlife is primarily limited to vertebrates, in which mechanistic support has been substantiated through a variety of in vitro and in vivo studies and laboratory animal studies (e.g. Luebke et al., 1997). Here lies perhaps the major impediment to advancing the concept of wildlife immuntoxicology and of ecotoxicology in general: while field and semi-field studies provide the best evidence for a 'real world' context, they generally fail to provide mechanistic evidence for the reasons listed above. A combination of research strategies entailing both field- and laboratory-based research has generally provided the most robust 'weight of evidence' for wildlife (**Figure 2.26**).

2.11.3.1 Marine mammals

Effects of EDCs on immune function in free-ranging marine mammals have been inferred from a number of studies, the most comprehensive of which were on the immunotoxic effects of contaminants in captive harbour seals (De Swart et al., 1994; 1995a; 1995b; 1996a; 1996b; Ross et al., 1995; 1996), in which a range of immunological parameters were measured in animals fed fish from the contaminated Baltic Sea over the course of 2.5 years. Differences in adaptive and innate immunity (such as natural killer cell activity and delayed-type hypersensitivity responses) were observed between experimental and control groups, with these differences attributed primarily to the PCBs in their diet.

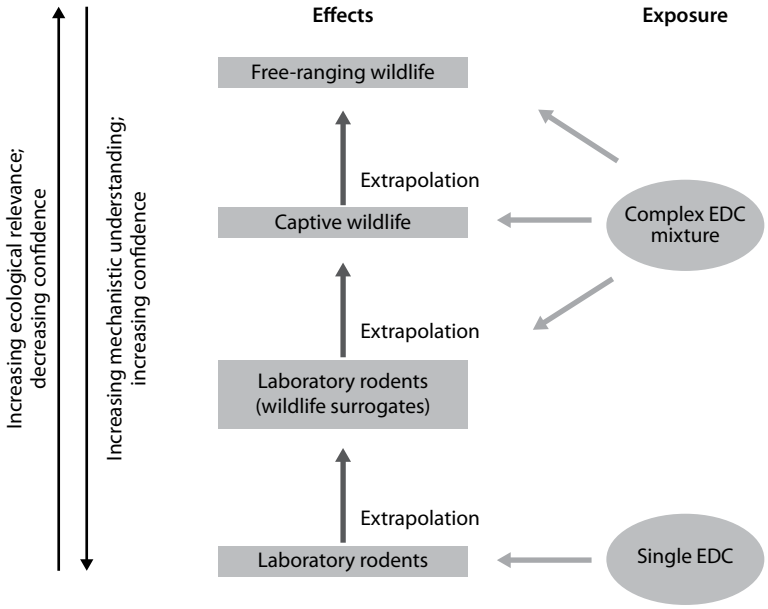

Figure 2.26. Since uncertainties exist for toxicological studies in free-ranging wildlife, assessment of EDC effects on the immune and other systems are best served by a "weight of evidence" from a combination of approaches. A wealth of toxicological information has been generated for many EDCs using laboratory rodent studies, but similar approaches are neither practical nor ethically acceptable in wildlife. In this manner, ecologically relevant information can be generated (i.e. free-ranging wildlife) that has a mechanistic basis in toxicology (i.e. laboratory rodent studies), and uncertainties can be minimized by appropriate and critical extrapolations among each of these lines of research (Modified from Ross, 2000).

Other studies also indicate associations between tissue concentrations of contaminants in marine mammals and changes in immune blood parameters, suspected of indicating immunotoxicity (Beckmen et al., 2003). Reduced mitogen-induced T-cell proliferation associated with elevated PCB and DDT blood levels was observed in free-ranging bottlenose dolphins off the coast of Florida, suggestive of a contaminant-induced inhibition of the cellular immune response (Lahvis et al., 1995). However, age may also have contributed to these results. Thymic atrophy and splenic depletion in 61 by-caught and stranded harbour porpoises were correlated with increased PCB and PBDE levels (Beineke et al., 2005). However, lymphoid depletion was also associated with emaciation and an impaired health status in these animals.

Mechanistic evidence for the effects of EDC exposure on immune function in marine mammals comes mainly from in vitro studies. For example, beluga whale splenocyte proliferative responses were reduced after exposure to a mixture of PCB and DDT congeners (De Guise et al., 1998). Organochlorines, individually and in mixtures, modulated in vitro respiratory burst (Levin, Brenda & De Guise, 2007), T-cell proliferation (Mori et al., 2006) and B-cell proliferation (Mori et al., 2008) in several cetacean species.

Despite the inherent challenges, evidence suggests that EDCs have affected immune function, thereby resulting in increased susceptibility to infectious diseases in vertebrates, notably marine mammals (Aguilar & Borrell, 1994; Hall et al., 1997; Jepson et al., 1999; Bennett et al., 2001; Jepson et al., 2005; Davison et al., 2011).

2.11.3.2 Birds

Immunological effects have been reported in captive male, but not female, American kestrels, exposed to PCBs, including an increase in total white blood cell counts amongst other responses (Smits et al., 2002). In an earlier study, Smits & Bortolotti (2001) also observed that PCB-exposed adult female American kestrels had a higher antibody response than did controls, whereas adult males exposed to PCBs had suppressed antibody production. These sex-specific responses in PCB-exposed birds provide further evidence of the endocrine disrupting behaviour of PCBs. These studies are further supported by those of Fernie et al. (2005) in which environmentally relevant concentrations of PBDEs modified cell-mediated and humoral immune functions in captive nestling American kestrels, and resulted in structural alterations in immune organs. In addition, there were alterations in the spleen (fewer germinal centers), bursa (reduced apoptosis) and thymus (increased macrophages). In wild glaucous gulls, the numbers of white blood cells were positively related to circulating concentrations of PCBs; Bustnes et al. (2004) and Sagerup et al. (2000) reported that glaucous gulls with high PCB and DDT levels had higher nematode burdens than individuals with low levels, suggesting an effect of these EDCs on host resistance. This evidence follows in the path of earlier pioneering work wherein ten-day-old mallard ducklings which had been fed PCBs exhibited higher mortality to duck hepatitis virus (Friend & Trainer, 1970).

2.11.3.3 Amphibians

Associations between EDCs and immunocompetence have been reported for amphibians, where a limited number of studies have shown that exposure to p,p'-DDT and PCBs correlated with poor immune responses (e.g. Gilbertson et al., 2003; Albert et al., 2007), parasitic infections and mortality events (Mann et al., 2009; for a review of wildlife exposure to POPs, see Chapter 3.2.1).

2.11.3.4 Fish

Immunological effects caused by exposure to environmental contaminants, including some EDCs, have been reported across a range of fish species, many of these related to aquaculture (reviewed by Wester, Vethaak & van Muiswinkel, 1994; Cuesta, Meseguer & Esteban, 2011). Planar PCBs are considered the most toxic as they bind and activate the Aryl hydrocarbon receptor (AhR) and cytochrome P4501A (CYP1A) expression, while non-planar congeners can interfere with AhR signalling but also affect cells via AhR-independent pathways (Duffy & Zelikoff, 2006). Interestingly, treatment of rainbow trout with 10-70% sewage treatment works effluent (containing PAHs among other contaminants) increased their in vitro lymphocyte proliferation but decreased the number of circulating lymphocytes (Hoeger et al., 2004). This effluent failed to alter other measured immune functions including respiratory burst, phagocytosis, lysozyme activity, leucocyte populations other than lymphocytes and *A.Salmonicida*-specific IgM production (Hoeger et al., 2004). In another study, however, exposure to a mixture of chemical contaminants (including some EDCs) caused impairment in the immune system that ultimately led to increased host susceptibility to infectious diseases (Arkoosh et al., 1998).

2.11.3.5 Invertebrates

Invertebrates represent about 95% of total species in the animal kingdom but only a few studies evaluating the effects of EDCs on the immune system have been done in these taxa (reviewed in Galloway & Depledge, 2001).

Molluscs

Different EDCs have been shown to affect immune function in marine bivalves (Canesi et al., 2003; Renault, 2011). Moreover, Porte et al. (2006) reviewed the effects and alternative mechanisms of action of natural and environmental estrogens in mussel (*Mytilus* sp.) immunocytes. Organotin compounds were found to be effective modulators of the immune system in molluscs (Fisher, Wishkovsky & Chu, 1990; Cooper et al., 1995; Cima et al., 1998; St.-Jean, Pelletier & Courtenay, 2002). Interestingly, contamination of the eastern oyster, by tributyltin increased the intensity of *Perkinsus marinus* infection, but no modulation of cellular or humoral parameters were detected

(Anderson, Unger & Burreson, 1996; Chu et al., 2002). An investigation on the effects of chronic exposure of eastern oyster to the insecticides DDT, toxaphene and parathion, noted the presence of an unidentified mycelia fungus in exposed oysters (Lowe et al., 1971).

Crustaceans

In marine crustaceans, there are a scarcity of data to support the notion that EDCs affect the immune system or alter their susceptibility to infectious disease agents. In one study, sub-acute concentrations of CB-15, but not CB-77, reduced haemocyte count but increased recoverable hemolymph volume (Smith & Johnston, 1992).

Echinoderms

The main immune responses of echinoderms are considered to be phagocytosis and the production of reactive oxygen species (ROS). These immune responses have been reported to be affected by EDCs. PCBs increased ROS production and delayed the timing of peak production in the echinoid *Paracentrotus lividus* (Coteur et al., 2001) but not in the sea star, *Asterias rubens* (Coteur et al., 2003). Butyltins affected the phagocytic activity of the amoebocytes of the polar seastar *Leptasterias polaris* (Békri & Pelletier, 2004).

Tunicates

Previous research has shown that in vitro exposures of the colonial ascidian, *Botryllus schlosseri*, to tributyltin and dibutyltin (1 μM) inhibited phagocytosis. Furthermore, exposure to monobutyltin (10 μM) resulted in a slight but significant decrease of this activity (Cima et al., 1995; 1997). The immunotoxicity of organotins, reflected by altered phagocytic activity, chemotaxis or ROS generation, have also been identified in the tunicates, *Styela plicata* (Raftos & Hutchinson, 1997) and *Ciona intestinalis* (Cooper et al., 1995)

Annelids

The commercial PCB mixture Aroclor 1254 suppressed natural cytotoxicity (Suzuki et al., 1995) and phagocytosis (Burch et al., 1999) in the coelomocytes of the earthworm *Lumbricus terrestris*. Exposure in vivo to Aroclor 1254 also decreased host resistance to subsequent challenge of *L. terrestris* with the bacterium *Aeromonas hydrophila* (Roch & Cooper, 1991).

2.11.4 Evidence for a common mechanism of endocrine-immune diseases and disorders in human and wildlife species

The basic similarity in the immune systems of humans and other vertebrates has been used in support of an extensive history of toxicological and pharmacological research. The main function of the immune system in all organisms is to provide protection against infectious agents. Two basic defence systems have evolved in biota: the innate immune system (natural immunity) and the adaptive immune system (acquired immunity). The innate system can be found in all multicellular organisms (Hoffmann et al., 1999), while the acquired system is only found in vertebrates (Du Pasquier, 2001).

The AhR is found in diverse species, including mammals, birds, fish and invertebrates and, in addition to its poorly understood role in xenobiotic processing, it serves to modulate immune modulation in humans and laboratory rodents. The AhR provides a defensible mechanistic basis for extrapolation of toxicological and immuntoxicological effects associated with dioxin-like compounds. Contaminants in the Baltic Sea herring (containing PCBs among other contaminants) fed to laboratory rats (Ross et al., 1997) and harbour seals (De Swart et al., 1994; 1995a; Ross et al., 1996) caused a similar pattern of immunotoxicological effects in the two species, supporting a common mediation of this toxicity, and lending support to the notion that extrapolation from surrogate species can be used. Freshwater amphibian and fish immune function is reduced by ecologically relevant concentrations of atrazine, and this is regularly accompanied by elevated infections (reviewed by Rohr & McCoy, 2010). Similarly, both direct in vitro and in vivo exposure to atrazine decreases immune function (reviewed by Rowe, Brundage & Barnett, 2008) and disease resistance (Karrow et al., 2005) in rodents.

Hotchkiss et al. (2008) noted the sometimes controversial nature of associations between EDC exposures and adverse effects in humans, but the effects of PCBs on immune function is one case where the data are robust enough to support a cause and effect relationship (reviewed by Brouwer et al., 1999; Selgrade, 2007). Furthermore, animal laboratory studies corroborate many of these adverse effects observed in the field and, in some cases, provide mechanisms to explain the effects. Selgrade (2007) concluded that "suppression of immune responses in rodents is predictive of suppression of immune responses in humans and that there is a relationship between immune suppression following developmental exposure to the toxicants and enhanced risk of infectious or neoplastic disease in humans" for PCBs.

It is now evident that a 'weight of evidence' approach provides the best avenue for establishing causation in the case of complex EDC mixtures and effects on the endocrine and immune systems of wildlife (Ross, 2000). Laboratory-based screening protocols certainly provide the most cost-effective and mechanistically-based approach to predicting the effects of EDCs in the real world. However, continued vigilance in the field is also needed.

2.11.5 Main messages

- It is increasingly clear from both data in the laboratory and from human and wildlife samples that EDCs play a role in the development of immune-related disorders and are at least partially responsible for the rise of many of these diseases in recent years.
- Allergies, asthma, and airway disorders, endometriosis, and autoimmune thyroid disease in humans may have roots in EDC exposure.

- Systemic inflammation, immune dysfunction and immune cancers such as lymphoma and leukemia in humans have been connected to EDCs. These chemicals may exert their effects through nuclear receptor signaling pathways that have well-established ties with the immune system through crosstalk with inflammatory pathways. They may also interact with non-nuclear membrane-bound steroid receptors, might affect levels of endogenous steroids, and could work through both the HPA and HPG axes.
- Despite multiple inherent challenges, great strides have been made in establishing cause-and-effect linkages between exposure to EDCs and adverse effects in wildlife.
- During the last decade, a number of wildlife studies have shown that, in addition to their well documented role in regulating reproductive function, estrogens and androgens also modulate the immune system.
- Wildlife immunotoxicology may provide an indication of contamination and shed light on the mechanisms of endocrine disruption for some chemical compounds.
- Studies of caged fish and molluscs, as well as laboratory-based exposures, confirm that sewage effluent modulates the function of the immune system in some freshwater species.

2.11.6 Scientific progress since 2002

- The molecular mechanisms connecting a variety of nuclear hormone receptors to NF-kB (one of the master regulators of inflammation and immunity) have been elucidated.
- Exposure to phthalates and dioxin-like compounds have been linked with the development of endometriosis.
- Developmental immunotoxicity (DIT) has been further explored, linking compounds such as diethylstilbestrol and genistein to postnatal immune disorders.
- Links between PVC products and airway disorders and asthma have been expanded and strengthened.
- Triphenyltin has been linked by several studies to neutrophil and natural killer cell abnormalities.
- Animal data indicate that embryonic exposure to estrogens leads to prostate inflammation in the resulting offspring.
- New data connect bisphenol A to inflammation, allergic sensitization, increased antibody production, and Th2 immune responses.
- Atrazine has been found to affect both natural killer cells and T cells.

2.11.7 Strength of evidence

There is sufficient evidence that EDCs play a role in the development of immune-related disorders and are at least partially responsible for the increase in these diseases in recent years. Further epidemiological and laboratory investigation is warranted to connect correlative data on exposure with underlying causative mechanisms responsible for disease etiology.

There are substantial data linking systemic inflammation, immune dysfunction and immune cancers such as lymphoma and leukemia with EDCs. Together, these new insights stress a critical need to acquire a better understanding of how EDCs affect normal immune function and immune disorders, how windows of exposure may affect disease incidence (particularly for childhood respiratory diseases), and how these effects may be passed on to generations to come.

There is good evidence that EDCs acting through nuclear hormone receptor pathways can directly affect the HPA axis, particularly through their actions on the adrenal glands (Marx et al., 1998).

There are good epidemiological data linking PAH, PCBs and other persistent POPs with autoimmune thyroid disease (Burek & Talor, 2009; Guarneri & Benvenga, 2007; Langer et al., 2008). The mechanistic basis of these associations requires further investigation and offers opportunities for new research directions.

There are sufficient data linking exposure to phthalates, PCBs and dioxins with endometriosis (Cobellis et al., 2003; Heilier et al., 2005; Porpora et al., 2006; Reddy et al., 2006). Further investigation into the mechanistic basis of these links will be important for the future.

There are strong links between EDC exposure, particularly phthalates, and the rising incidence of asthma throughout the developed world (Bornehag et al., 2004; 2005; Kolarik et al., 2008). These links are also supported by animal studies (Bornehag & Nanberg, 2010), suggesting that the links are causal and should be explored in detail in the future.

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2.12 Endocrine disruptors and population declines

2.12.1 Trends in wildlife populations

It is clear that many wildlife populations have declined or are declining in numbers. There is worldwide concern about the loss of species or reduced populations of amphibians, mammals, birds, reptiles, freshwater and marine fishes, and invertebrates (Myers & Worm, 2003; Butchart et al., 2004; Stuart et al., 2004; Clausnitzer et al., 2009; Cumberlidge et al., 2009; Hoffmann et al., 2010; 2011; Vié, Hilton-Taylor & Stuart, 2009; Vorosmarty et al., 2010) (**Figure 2.27**). These declines are more severe in some regions than others, but most have been linked to human activities such as direct harvesting (over-exploitation), and development resulting in habitat loss, environmental contamination, or global climate change (e.g. Myers & Worm, 2003; Hayes et al., 2010; Zhou, Cai & Zhu, 2010). These activities have directly or indirectly affected the ability of many species to survive and reproduce, both of which are critical for a healthy population.

While there is strong evidence that EDCs are affecting the survival and reproduction of individuals (Cheek, 2006; Milnes & Guillette, 2008; Hamlin & Guillette, 2010, 2011; Letcher et al., 2010), making the link between these effects and changes to the population numbers or biodiversity in a region is much more difficult. The natural abundance of fishes, mammals, reptiles, amphibians, birds and invertebrates is often not well understood, but can be affected by chemical exposure (through direct toxicity and endocrine mechanisms), resource extraction, the availability of resources (food, habitat), and competition from or predation by other species. Understanding the role of exposure to EDCs in the decline of species or biodiversity in a region is challenging because of the presence of other natural or human stressors, the mixtures of chemicals (both EDCs and non-EDCs) that many populations are exposed to and the difficulty in assessing exposures (see Chapter 3), and our limited understanding of the ecology of the population. Declines in the abundance of one species will in turn affect the health and balance of its ecosystem because of the interdependencies of organisms within the environment.

2.12.2 Evidence for EDCs causing population declines in wildlife

The best evidence of EDCs causing declines in wildlife species has been from associations between exposure to a chemical with known impacts on the endocrine system (mainly from lab studies) and changes in the numbers of wild animals. As chemical exposures increase, populations decline; conversely, as chemicals are removed from the market and wildlife exposure decreases, populations recovers. The two best examples of these types of relationships are for the pesticides DDT and tributyltin (TBT).

2.12.2.1 Tributyltin and invertebrate populations

TBT is an anti-fouling compound that was common in paints used on ships in the 1970s through to the 1990s. Exposure to TBT caused imposex (development of male sex organs) in female snails and led to reproductive failure and declines or extirpations of several species in harbours and other areas with high TBT use (Titley-O'Neal, Munkittrick &

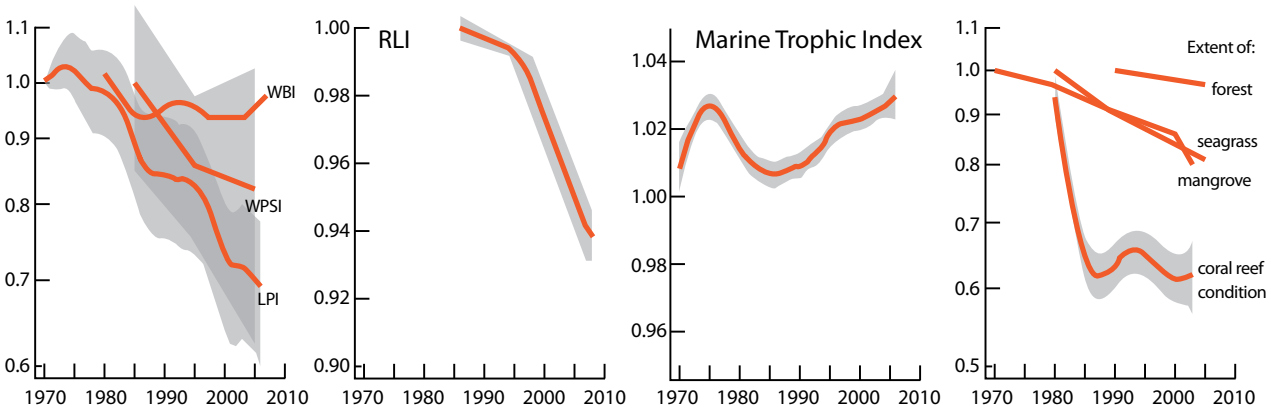

Figure 2.27. Indicator trends for the state of biodiversity. Data scaled to 1 in 1970 (or for first year of data if >1970), modeled, and plotted on a logarithmic ordinate axis. Shading shows 95% confidence intervals except where unavailable (i.e. mangrove, sea grass, and forest extent). WBI, Wild Bird Index (The Global Wild Bird Index aims to measure population trends of a representative suite of wild birds for which robust data are available, to act as a barometer of the general health of the environment and how it is changing), WPSI, Water Bird Population Status Index (similar to WBI but only for water birds and focused on direction of change in population rather than magnitude of change), LPI, Living Planet Index (state of biological diversity for vertebrates around the world. Derived from the ***Living Planet Database (LPD)*** which contains over 10,000 population trends for more than 2,500 species of fish, amphibians, reptiles, birds and mammals), RLI, Red List Index (The ***Red List Index (RLI)***, based on the IUCN Red List of Threatened Species, is an indicator of the changing state of global biodiversity. It defines the conservation status of major species groups, and measures trends in extinction risk over time). Figure based on Butchart et al. (2010) and reproduced with permission from the publisher.

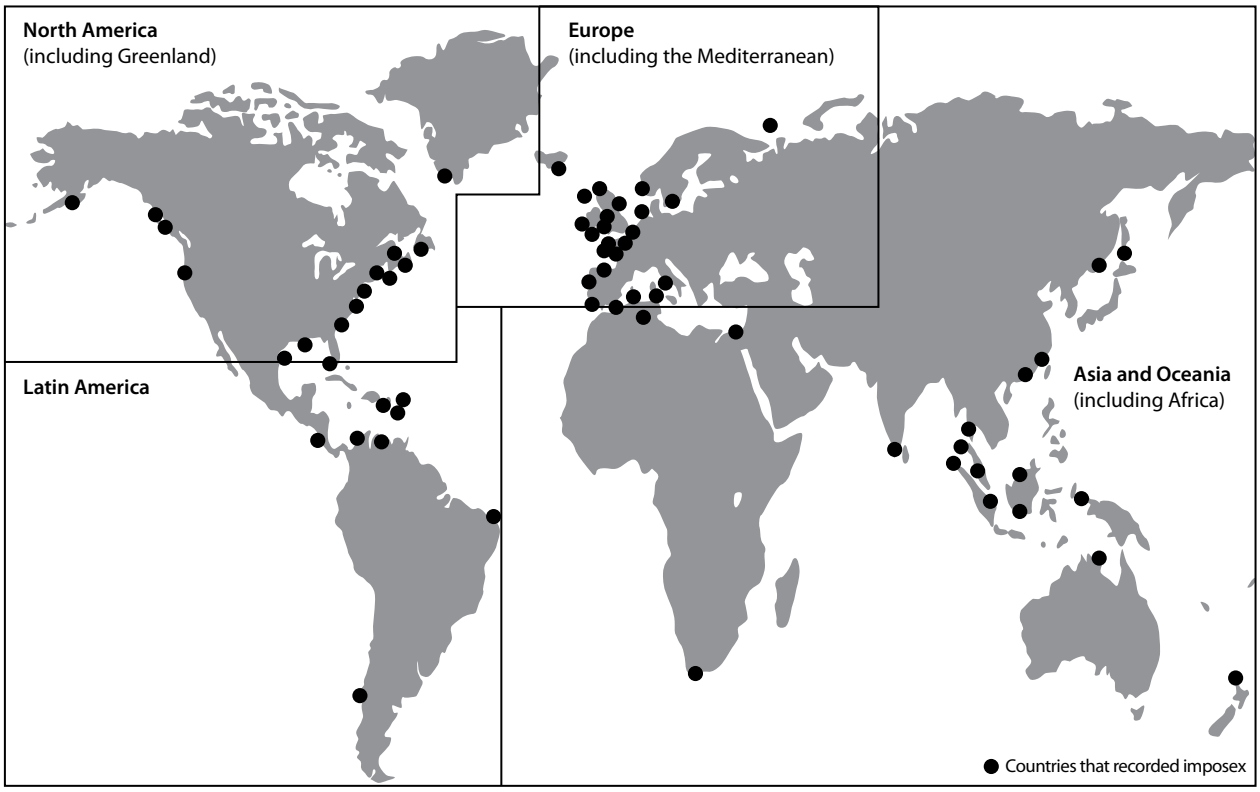

Figure 2.28. Geographic regions of the world where female gastropods were reported as affected by imposex, intersex and ovo-testis between 1990 and 2009 (Titley-O'Neal et al., 2011). Figure based on data from the reference given.

MacDonald, 2011; **Figure 2.28**). The use of TBT has been restricted (but not banned completely worldwide) since the 1990s and, as a result, snail populations have recovered in locations where environmental concentrations of TBT have declined (Jorundsdottir, Svavarsson & Leung, 2005; Morton, 2009; **Figure 2.29**). Similarly, other invertebrates also historically affected by TBT have shown recent signs of recovery in abundances, although an endocrine disrupting mechanism has not been identified. For example, the populations of North Sea brown shrimp declined steeply prior

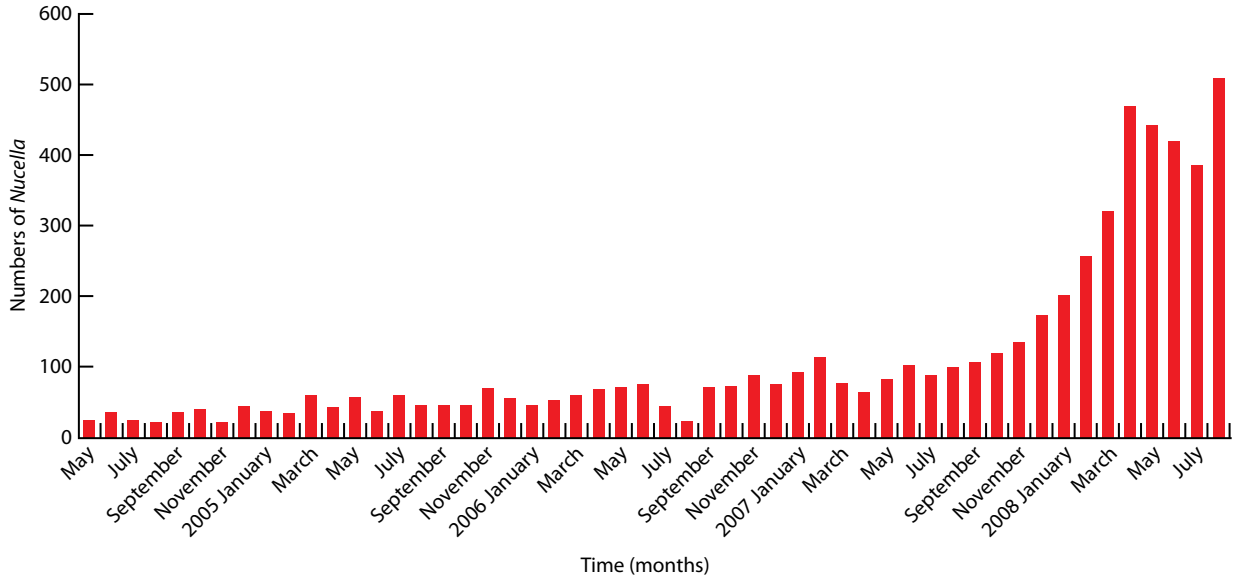

Figure 2.29. The numbers of dog whelks (*N. lapillus*) recorded from a single location (Mewsbrook Groyne at Littlehampton on the southeastern coast of England) every month from May 2004 to August 2008 coinciding with the period immediately after TBT was banned globally as a ship anti-foulant (Morton, 2009). During the study period, the size of the population of *N. lapillus* grew from ~25 individuals to >500, i.e., a 20-fold increase. (Figure redrawn from Morton, (2009); Used with publisher's permission)

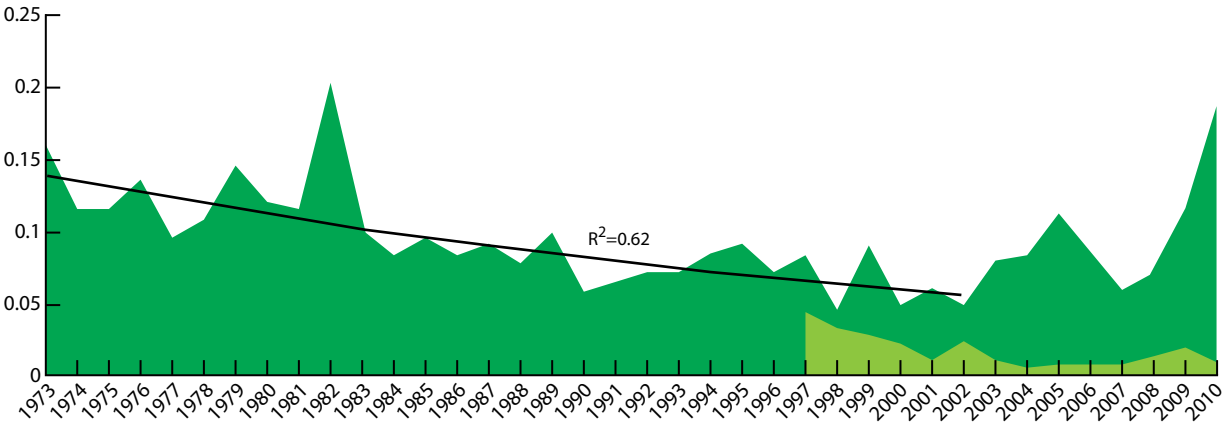

Figure 2.30. Effects of TBT on the North Sea brown shrimp (*Crangon crangon*) stock prior to and since the TBT ban in 2003. Long-term annual time series of landings per unit effort (LPUEs) for the Southern Bight (Y-axis, average monthly LPUEs in kg FW x horsepower-1 x fishing hours-1) for the North Sea brown shrimp (dark green) and cod and whiting (light green) during 1973–2010 and 1997–2010, respectively (X-axis). (Figure redrawn from Verhaegen et al. (2012); Used with publisher's permission)

to an EU ban in 2003, and corresponded with accumulation of unacceptably high levels of TBT in their tissues, such that they could not be consumed by people. Levels have decreased approximately 10-fold since the ban took effect, coinciding with a recovery of the shrimp stock after 30 years of gradual decline (**Figure 2.30**; Verhaegen et al., 2012).

2.12.2.2 Organochlorine pesticides and bird populations

Similar population declines and recoveries for top predator bird species have been observed with the heavy use and subsequent restriction of the insecticide DDT. In the environment, DDT is broken down into DDE, a form that concentrates up through the food web to elevated levels in upper-trophic-level birds such as osprey, falcons and eagles, and this chemical interferes with the hormones (prostaglandin signalling) controlling eggshell production. As DDE exposure increased, eggshell thickness decreased in birds worldwide (Hickey & Anderson, 1968; Grove, Henny & Kaiser, 2009; Blus, 2002), affecting both the survival of the chick and the reproductive success of the species. When DDT was heavily used in North America and Europe, numerous bird populations (e.g. brown pelicans, merlins, double-crested cormorants, great cormorants, peregrine falcons, bald eagles, osprey) with high DDE exposure declined because they were unable to successfully reproduce (Blus, 2002). Field monitoring programs were invaluable in linking the declines to DDE (Blus & Henny, 1997). Populations of some of these birds have recovered or are recovering due to bans on the use of DDT in many countries. As an example, after DDT was banned in North America in 1972, osprey populations in the US increased from 8000 in 1981 to 14 200 in 1994 to 16 000-19 000 in 2001 (Grove, Henny & Kaiser, 2009). There is a clear association between the DDT ban (as evidenced by declining levels of DDE in osprey eggs) and the recovery of this species (**Figure 2.31**; Henny et al., 2010). This raises the possibility that avian species where

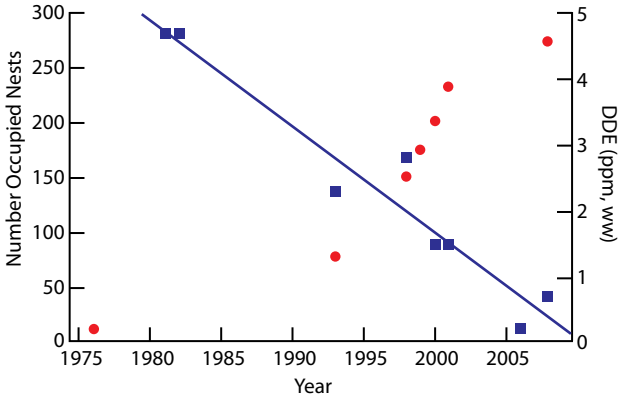

Figure 2.31. Number of osprey nests occupied (left Y axis; circles) versus DDE concentrations (squares; right Y axis; geometric means, ppm wet weight, and best fit line) in osprey eggs from Willamette River, Oregon, USA. Figure based on data from Henny et al. (2010).

DDT is still used for malaria control may be adversely affected. Bouwman et al. (2008) found several POPs (HCB, DDT, HCHs, chlordanes and PCBs) at detectable levels in eggs from 8 bird species in South Africa. Of those, the African darter had thinner eggshells that were associated with higher egg concentrations of DDE, PCBs and other POPs. Monitoring the impact of DDT on birds is essential in areas where ongoing spraying occurs.

2.12.2.3 Mercury and bird populations

There is ongoing concern about exposures of birds to methylmercury - the form of mercury that biomagnifies up through food webs to high concentrations in top predators - and how it affects populations. For example, concentrations of mercury in eggs of ivory gulls collected from Seymour Island, Canada, have steadily increased since 1976 to levels which are

Table 2.8. Nesting effort of white ibises and body feather mercury (Hg) concentrations in great egret chicks in the Florida Everglades. The number of white ibis nests are negatively correlated with great egret feather mercury concentrations (Spearman-rank rs = −0.77, p = 0.04). Data are from a seven-year standardized dataset of great egret chick-feather mercury concentrations (as a measure of temporal changes in mercury bioavailability) and annual numbers of white ibis nests (Heath & Frederick, 2005). (Table reprinted with permission of the publisher).

Year	Maximum number of White Ibis nests	Mean Great Egret feather Hg
1994	5182	17.40
1995	8177	11.48
1997	5989	19.10
1998	4971	8.67
1999	14014	7.36
2000	32204	5.96
2001	13144	8.35

now among the highest measured in seabirds, and these high concentrations appear to have had a long-term effect on breeding productivity (Braune, Mallory & Gilchrist, 2006; Gilchrist & Mallory, 2005). Similar concerns have also been reported in other bird species. The mechanisms of endocrine impairment and net effects on demography of bird populations are poorly understood. However, a recent long-term lab study mimicked the dietary methyl-mercury exposure (0.05–0.3 ppm wet weight) of wild ibises and found increases in male–male pairing behaviour (to 55% of males), and decreases in egg productivity (to 30%) and fledgling production (to 35%) in captive ibises (Frederick & Jayasena, 2011). Endocrine disruption caused by mercury exposure could, therefore, lead to altered demographic patterns in wild bird populations (e.g. Burgess & Meyer, 2008). Indeed, Heath & Frederick (2005) suggest that breeding population size of ibises in the Florida Everglades may be inversely correlated with annual methyl mercury exposure (**Table 2.8**).

2.12.2.4 PBDEs

PBDEs are found at elevated concentrations in birds, especially those that are high in the food web and those near urban centers (Chapter 3.2). Lab studies have shown that reproduction is compromised in American kestrels exposed to environmentally relevant concentrations of PBDEs in their diet. More specifically, these birds had delayed times to egg laying, and laid eggs that had thinner shells and smaller weights (Fernie et al., 2009). Although no field studies have linked raptor abundances with PBDE exposure, lab studies like this suggest that populations of wild birds accumulating high concentrations of PBDEs may also be compromised.

2.12.2.5 Fish populations and estrogens

Understanding the links between EDCs and fish abundances downstream of point source inputs of municipal or industrial effluents or in systems receiving non-point source contamination (e.g. runoff from agriculture) is challenging mainly because EDC exposure is one of several stressors in the aquatic environment. However, population modeling based on lab studies has predicted declines in the abundance of fishes exposed to low, environmentally relevant concentrations of estrogens and their mimics (e.g. Grist et al., 2003). A long-term, whole-lake experiment showed dramatic declines in the fathead minnow population after exposures to low ng/L concentrations of EE2 (Kidd et al., 2007). Male fish have high incidences of intersex in rivers receiving sewage treatment works effluents that contain estrogens and anti-androgens (e.g. roach in the UK; Jobling et al., 2006), and this condition decreases their reproductive success when in competition with normal males (Harris et al., 2011). EDCs are likely impacting fish abundance and genetic diversity (see below), but these impacts are difficult to detect and, hence, our ability to link EDCs directly to population declines for fishes remains an ongoing challenge (Mills & Chichester, 2005; Sumpter & Johnson, 2008).

2.12.2.7 Marine mammals

Several studies have suggested a role for chemical exposure in the decline of marine mammal populations through their effects on reproduction and survival. Although the evidence is speculative due to the difficulty of associating the chemicals directly to lower numbers of individuals, declines in the sea otter, northern fur seal, Steller sea lion and the Galapagos sea lion may be partially due to their exposure to diverse mixtures of PCBs, DDT, other POPs, mercury and other metals (Alava et al., 2011; Barron, Heintz & Krahn, 2003; Beckmen et al., 2003; Kuker & Barrett-Lennard, 2010; Towell, Ream & York, 2006). The numbers of Baltic grey seals declined in the late 1970s to less than 4 000 individuals from an estimated 88 000 to 100 000 animals 100 years earlier (Harding & Härkönen, 1999). More recent population estimates indicate around a 7% increase in the populations per year likely due to lower POPs exposures in

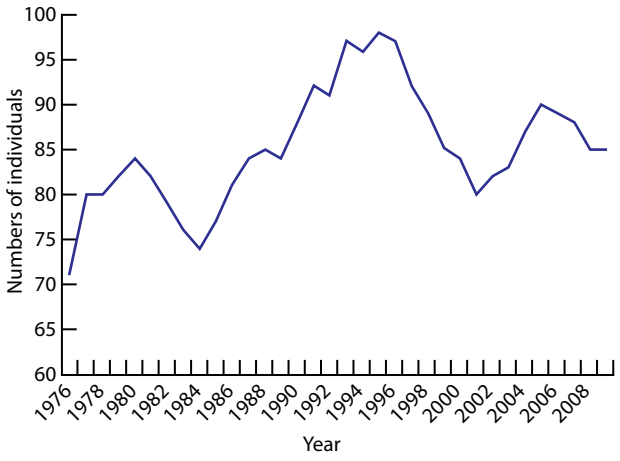

Figure 2.32. Abundance of southern resident killer whales from 1976-2009 (data from the Center for Whale Research) (Pudget Sound Partnership (2009); redrawn; Used with publisher's permission)

the last decade (Olsson, Karlsson & Ahnland, 1994; Karlsson et al., 2005). The southern resident killer whales (SRKW) also experienced an unexplained 20.4% decline in their population between 1995-2001 (see **Figure 2.32**) (Centre for Conservation Biology, 2012). This is an alarming rate of decline for an already small population; the SRKW represents the smallest of four resident communities (consisting of only 89 individuals in three pods) within the eastern North Pacific Ocean and is the only killer whale population to be listed as endangered.

There are three current hypotheses that are posed as reasons for the decline in the SRKW: 1) a decline in the whales' primary prey, Chinook salmon; 2) noise disturbance from private and commercial whale watching vessels; and 3) exposure to high levels of endocrine disrupting POPs (e.g. PCBs, PBDEs and DDT), which are stored in the whales' fat. Because they are long-lived top predators, killer whales accumulate high concentrations of POPs, including PCBs and PBDEs. Killer whales are known to be the most contaminated marine mammals in the world, due to the high levels of toxic anthropogenic chemicals that accumulate in their tissues (O'Neill & West, 2009; Hickie et al., 2007). PCB concentrations measured in biopsies collected from killer whales (Ross et al., 2000; Krahn et al., 2009) exceed the effects threshold established for harbour seals (17 mg/kg PCBs in blubber; see section 2.2) by several times (Kannan et al., 2000; Ross et al., 1996). As PCBs restrict the development of the reproductive system in cetaceans (see Chapter 2.2), high contamination levels lead to low pregnancy rates and endocrine and immune system disruption; both systems are critical to mammalian health and survival (Ross et al., 2000). These or other contaminants may be a factor in the decline of endangered populations of killer whales (Ross, 2006; Krahn et al., 2004), although the mechanism via which these effects may occur is unknown. In a very recent study (Buckman et al., 2011), biopsies from the SRKW tissues revealed that PCB tissue loads were strongly correlated with increases in the expression of aryl hydrocarbon receptor, thyroid hormone α receptor, estrogen α receptor, interleukin and metallothionein 1, thus providing the first evidence of endocrine disruption related to exposure to PCBs in these animals. Reduced exposure to these contaminants will be important to the recovery and long-term survival of the southern resident killer whales and of other killer whale populations.

2.12.3 Evidence that EDCs cause declines in genetic diversity in wildlife populations

Declines in the abundance or extirpation of a species is the most severe response to EDC exposure but there are other more subtle but potentially devastating impacts on wildlife populations over the long term. EDCs could also impair reproduction and an individual's ability to contribute genetically to the next generation (fitness). Chemical exposure is known to decrease the genetic diversity of populations either directly because of mutations to the DNA (genotoxic effects) or indirectly because only the tolerant individuals survive and reproduce, reducing the genetic information in the next generation (Medina, Correa & Barata, 2007; Bickham, 2011). It is likely that this also occurs in response to EDCs because of their impacts on reproductive success of the parents and survival of the offspring. For example, laboratory exposure of zebra fish to a potent pharmaceutical estrogen, EE2, changed the genetic composition of the offspring because it reduced the spawning success of some individuals and increased the success of others (Coe et al., 2008). Estrogen exposure in male fish has also been shown to cause chromosomal abnormalities (aneuploidy) in sperm and in the embryos they fertilize (Brown et al., 2008). Reduced genetic diversity in a population could threaten its ability to survive changes in the environment, increase its risk of extinction, and may in turn impact how communities and ecosystems function (Medina, Correa & Barata, 2007). At a higher level of biological organization, for example, it has been reported that marine communities have lower biodiversity in polluted than in non-polluted areas (Johnston & Roberts, 2009). Although a direct link between EDC exposure and reduced genetic diversity in populations or communities has not yet been demonstrated, it is possible that this may occur given the effects of EDCs on the reproductive success of wildlife.

2.12.4 Human populations

Increasing human populations in many places in the world have been of major concern due to their impact on food and energy resources. Therefore, since the 1960s major national and international bodies, including the Population Council and WHO, have been operating large family planning programs (Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, 1211 Geneva 27, Switzerland). These programmes seem to have

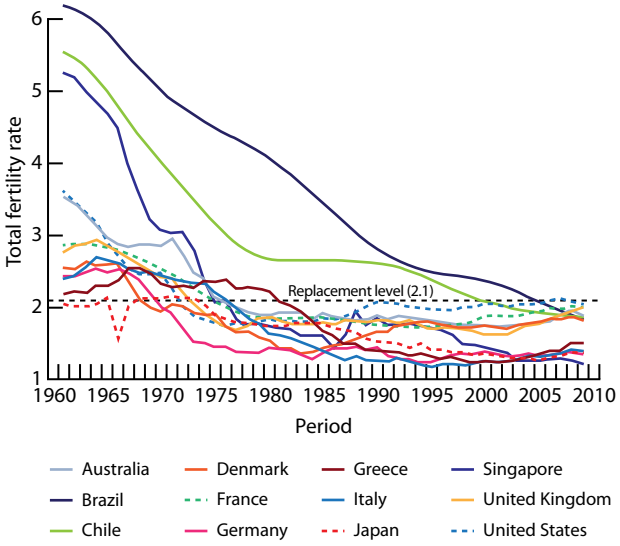

Figure 2.33. Fertility (1961-2009) in countries where current fertility rates are below replacement level. Diagram based on data presented by the World Bank (http://data.worldbank.org/indicator/SP.DYN.TFRT.IN).

been successful. Besides contributing to reductions in fertility rates (see **Figures 2.33 and 2.34**), they have also resulted in improved economic status of individuals and resulted in worldwide improvement in women's reproductive health and social status in general. China took part in this development by introducing the one child family programme in the 1970s.

In spite of the drastic reductions in fertility rates are still increasing. There are two main reasons for this. The first is that, although fertility rates seem to be declining all over the world, there are still areas, particularly in developing countries, where fertility is significantly above the replacement level (an average of 2.1 children per woman) (**Figure 2.33**). Somalia and Tanzania are examples of African countries with high fertility and it is noteworthy that all high fertility countries belong to less industrialized areas (**Figure 2.34**). The second main reason for the current increase in the world population is that changes in fertility rates are not completely reflected in population statistics until 30-60 years after they occur. The reason is that humans live much longer than before due to improved health conditions. Therefore today we see rather stable, but ageing, human populations in countries like Japan and Europe, where fertility rates have been below the replacement level for 20-40 years. However, we shall soon begin to see significant reductions in populations of these industrialized countries, although immigration may modify this development.

It has generally been believed that the low fertility rates have been due to contraception combined with political initiatives (China) or changes in social family structures (Japan, South Korea, Singapore, Chile, Europe, Australia) (Lutz, 2006; UN DESA, 2011). Reproductive health problems among men and women may also be important factors behind the low fertility rates (Jensen et al., 2008). In countries like Denmark where health statistics are well developed, there are accurate figures of the use of assisted reproduction techniques (ART); in Denmark, 8% of all children are now born after ART, including in vitro fertilisation, intra-cytoplasmatic sperm injection, intrauterine insemination by partners' sperm and intrauterine insemination by donor sperm (European Science Foundation, 2010). In spite of the common practice of ART, almost 25% of men are still childless (voluntary or involuntary). These numbers, taken together with recent data showing that poor semen quality is widespread (20%-40% of young men have suboptimal semen quality) (Jørgensen et al., 2012), suggest that reduced fecundity may also play a role in the current low fertility rates in Denmark. Trends in conditions causing low female fecundity have been less well examined (Crain et al., 2008). However, it is assumed that male and female factors contribute equally to human infertility, suggesting that female infertility may also play a role in these current fertility trends (see also section 2.2).

As human reproduction is a very slow process (30-40 years between generations) compared to many animal species, adverse trends in reproduction may not have a full population effect until after one or two generations. In other words, the low fertility rates we are witnessing today will not have full societal effect in our time. The European Science Foundation (2010) has recently highlighted the evidence that environmental factors play a role in adverse trends in male reproductive problems (testicular cancer, poor semen quality, low testosterone levels and other genital abnormalities; see also section 2.3) and urged both its European members and global research bodies to take part in the endeavor to identify the causes of the adverse environmental reproductive trends.

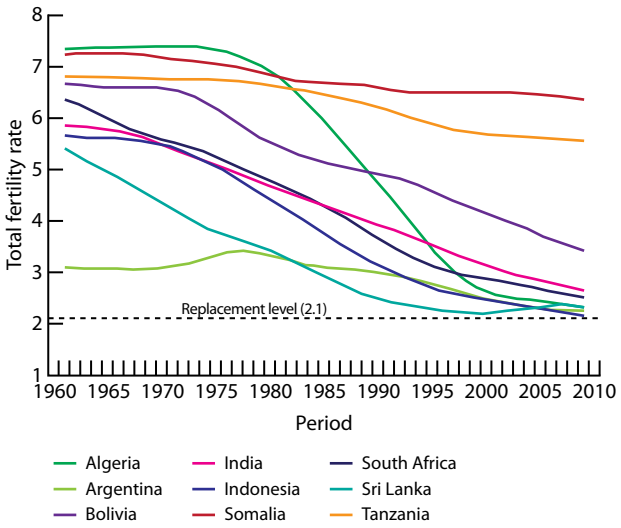

Figure 2.34. Fertility (1961-2009) in countries where current fertility rates are above replacement level. Diagram based on data presented by the World Bank (www.worldbank.org), from http://data.worldbank.org/indicator/SP.DYN.TFRT.IN.

2.12.5 Main messages

- Wildlife species and populations continue to decline worldwide and this is due to a number of factors including over-exploitation, loss of habitat, climate change, and chemical contamination.
- Although declines (and sometimes recoveries) in abundances of birds, marine mammals, fish, and snails have been related to changes in exposure to EDCs, making a clear link between endocrine effects in individuals and population declines is challenging.
- It is clear that historical declines in some wildlife populations (e.g. birds and snails) were because of the effects of chemicals (DDT and TBT, respectively) on the ability of these species to successfully reproduce. Bans on the use of these chemicals led to the recovery of some populations. For this reason, EDCs are strongly suspected to contribute to current declines in wildlife populations.
- In spite of concerns about rising human populations on a global scale, numerous industrialized countries have fertility rates well below the replacement level. It has generally been assumed that these changes are due to socioeconomic factors. However, widespread poor semen quality at subfertility levels may also contribute to this trend.

2.12.6 Scientific progress since 2002

In general, the evidence for endocrine disrupting POPs such as PCBs and OCs causing population declines has increased now, relative to 2002, due to the visible increases in populations of birds and seals, for example, following the restrictions on the use of these chemicals.

2.12.7 Strength of evidence

Wildlife Populations: While it is clear that the biodiversity and abundance of wildlife are threatened from a number of human activities, making direct links between declines in species and endocrine disruption from chemical exposure remains a major challenge. Relationships between exposure to EDCs and decreases in animal abundance or genetic diversity are correlative at best because of the difficulty in isolating effects of chemicals from other stressors (i.e. loss of habitat, overharvesting or climate change). To date the best evidence of a relationship between EDCs and wildlife populations is from a temporal relationship between a measure of exposure (e.g. DDT in bird eggs or TBT in snails) and population parameters (e.g. number of active bird nests or snails, respectively). When the species' exposure to the chemical declined through bans on its use, populations recovered. Current exposures to chemicals are compromising the endocrine system of many species and, as such, are believed to be playing a role in the lower abundances of wildlife.

To date, the quality and strength of the evidence linking EDC exposure to most wildlife population declines is insufficient. An endocrine mechanism for wildlife declines is probable but not conclusive.

Human Populations: Similarly, endocrine mechanisms linking EDCs to steep declines in fertility rates, such that in many countries they are below replacement levels, are plausible, but not explored.

2.12.8 References

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Chapter 3

Human and wildlife exposures to EDCs

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3.0 Introduction

A decade ago, the first IPCS *Global Assessment on the State-of-the-Science of Endocrine Disruptors* (IPCS, 2002) focused mainly on a few environmentally persistent organic pollutants (POPs; primarily PCBs, DDTs, PCDFs/PCDDs), with only very brief descriptions of other chemicals believed to contribute to endocrine disruption (ED) such as phytoestrogens and the flame retardants, polybrominated diphenyl ethers (PBDEs). The limited scope of this first report was primarily because there was much less information about the extent to which other chemicals might affect the endocrine system and even less information about exposure to these chemicals.

Over the past decade, understanding of the types of chemicals that may be endocrine disruptors and on how humans and wildlife are exposed to them has increased dramatically. A larger number of chemicals belonging to diverse classes are now identified as EDCs, and they include additives in materials and consumer goods (pharmaceuticals, personal care products, electronics, food packaging, clothing, etc.), metals, and current-use pesticides (**Table 3.1**). These chemicals come from a variety of sources, enter the environment during production, use or disposal of chemicals or products, and have a range of behaviours in the environment.

Over the last decade, some important exposure-related issues have emerged for EDCs. Now there is a better understanding that exposure to even very low concentrations of EDCs can increase the risk of effects (Chapter 1). Further, it is also understood that there are additional, important sources of exposure to EDCs, including dust from indoor environments and direct contact with consumer products. Concerns for the fetus and for young children has increased because, for some sources of EDCs, exposure is greater for young children (i.e. dust and particulates) than for adults (Lunder et al., 2010; Wormuth et al., 2006), and there is a greater understanding of the enhanced susceptibility to EDCs during these early developmental periods (Woodruff et al., 2008; see also Chapter 2). It is also evident that both human and wildlife exposures to EDCs consist of complex mixtures of chemicals that are persistent (remain intact for many years) and bioaccumulative (concentrate in fat or protein) or less persistent and not bioaccumulative (rapidly excreted). However, there is limited understanding of the types of mixtures that humans and wildlife are exposed to and how they affect the endocrine system, even though combined exposures can result in a greater risk than exposure to any one chemical at a time (National Research Council, 2008). In addition, effects related to exposure to EDCs could be complicated by exposure to non-EDC chemicals and possibly to other environmental, biological or physical stressors. Indeed, these are the main reasons why the evidence linking exposure to chemicals with endocrine disruption seen in human and wildlife populations (reviewed in Chapter 2) are often not definitive; all disorders and diseases are probabilistic and multicausal.

This chapter describes the current knowledge on the types, sources, environmental fate, exposure routes, and levels of "known" and "potential" (or "possible") EDCs (see Chapter 1.3 for definitions) in humans and wildlife. Although some of the chemicals from the 2002 document (e.g. PCBs) are included both here and in Chapter 2, the focus in this document is on chemicals that have been more recently identified as or suspected to be EDCs and those that are still produced and used. The end of each sub-chapter contains the main conclusions and the chapter ends with the main messages.

3.1 The EDCs of concern

This chapter is not intended to be a comprehensive description of all EDCs, but it identifies and describes major classes of chemicals with known or potential ED properties, and example chemicals from each class (**Table 3.1**). The EDC classes are defined according to common chemical properties and structural features, and on their use and occurrence (**Table 3.1**). This does not mean that the chemicals described here have only endocrine disrupting properties, as several have other toxicological properties and/or health effects. In addition, some of the chemicals could be included with several different classes due to their broad uses but, for simplicity's sake, are only presented in one place. Known or potential EDCs have been identified based on several reviews or authoritative reports (US EPA 2009; 2010a; Kortenkamp et al., 2011; Ryu, Yoon & Oh, 2011; McKinlay et al., 2008; EEA, 2012; Pongratz & Vikström Bergander, 2011; ChemSec 2011; TEDX, 2011; Lintelmann et al., 2003). **Table 3.1** presents only a portion of the EDCs that have been identified in the scientific literature, and additional chemicals are listed in the above-mentioned reviews. It is of note that there are many chemicals described here to which we and wildlife are exposed, but for which there are very little or no epidemiological data or data from animal studies described in Chapter 2.

3.1.1 Types and sources of EDCs

Hundreds of individual anthropogenic and natural chemicals are known or suspected to interact with endocrine systems in

humans and wildlife, and their sources of exposure, chemical properties, and environmental fate vary widely. For the purposes of this report, the chemicals are grouped into eleven broad classes based on their physical-chemical characteristics or origin/application areas (**Table 3.1**). The classes include chemicals that are currently produced for commercial purposes, and those that are no longer manufactured or are being phased out of production. The previous assessment on EDCs was primarily on POPs (IPCS, 2002) but this has broadened more recently to include less persistent and less bioaccumulative organic chemicals (e.g. current-use pesticides, plasticizers, pharmaceuticals, natural hormones, phytoestrogens, product additives) and metals. Some EDCs are persistent in the environment, bioaccumulate through food webs to high concentrations in wildlife and humans, and can be transferred to the developing fetus and the newborn through the placenta or breast milk, respectively. Other EDCs are less persistent in the environment and do not remain in humans and wildlife for very long (short half-lives), as for bisphenol A with a half-life of 4-8 hours (Vokel et al., 2002); more specifically, they are not bioaccumulative yet they are a concern because exposure to them can be continuous. Of all of the chemicals on the market (slightly less than 145,000 chemicals are currently preregistered by REACH (ECHA, 2011)), large numbers are persistent enough to reach humans and wildlife but are not accumulated in the body. These chemicals have been classified as *pseudo persistent*, i.e. through continuous emission to the environment they essentially become "persistent" pollutants even if their half-lives are short (Daughton, 2003). Some chemicals affect the endocrine system in their original form, whereas others undergo metabolic transformations in the body or are abiotically transformed to forms that make them active in endocrine systems. Humans and wildlife are exposed to a diverse number of EDCs through a variety of routes and, as described below, the levels found in body tissues are affected by environmental and socioeconomic factors. Despite an improved understanding of the types of EDCs that are in the environment, there are still knowledge gaps.

In contrast to a decade ago, there is a better appreciation that industrial and consumer products can contain known or potential EDCs. For example, cosmetics and other personal care products (shampoos and other hair products, toothpaste, soaps, lotions) contain fragrances (e.g. galaxolide), solvents (e.g. cyclic methyl siloxanes), preservatives (e.g. parabens), plasticizers

Table 3.1. Endocrine disrupting chemicals (EDCs) can be grouped in multiple ways. In this table known or potential EDCs are grouped into 11 categories with examples of individual EDCs. Bolded chemicals were selected since they are regarded to be of specific interest as EDCs, and are described in more detail in the text.

Classification	Specific Examples of EDCs[1]
Persistent and bioaccumulative halogenated chemicals	
Persistent Organic Pollutants (POPs) (Stockholm Convention) (section 3.1.1.1)	PCDDs/PCDFs, **PCBs**, HCB, **PFOS**, **PBDEs**, PBBs, Chlordane, Mirex, Toxaphene, **DDT**/DDE, Lindane, Endosulfan
Other Persistent and Bioaccumulative Chemicals (section 3.1.1.2)	**HBCDD,** SCCP, PFCAs (e.g. **PFOA**), Octachlorostyrene, PCB methyl sulfones
Less persistent and less bioaccumulative chemicals	
Plasticizers and Other Additives in Materials and Goods (section 3.1.1.3)	Phthalate esters (**DEHP**, BBP, DBP, DiNP), Triphenyl phosphate, Bis(2-ethylhexyl)adipate, n-Butylbenzene, Triclocarban, Butylated hydroxyanisole
Polycyclic Aromatic Chemicals (PACs) including PAHs (section 3.1.1.4)	**Benzo(a)pyrene**, Benzo(a)anthracene, Pyrene, Anthracene
Halogenated Phenolic Chemicals (HPCs) (section 3.1.1.5)	2,4-Dichlorophenol , Pentachlorophenol, Hydroxy-PCBs, Hydroxy-PBDEs, Tetrabromobisphenol A, 2,4,6-Tribromophenol, **Triclosan**
Non-halogenated Phenolic Chemicals (Non-HPCs) (section 3.1.1.5)	**Bisphenol A**, Bisphenol F, Bisphenol S, Nonylphenol, Octylphenol, Resorcinol
Pesticides, pharmaceuticals and personal care product ingredients	
Current-use Pesticides (section 3.1.1.6)	2,4-D, **Atrazine**, Carbaryl, Malathion, Mancozeb, **Vinclozolin**, Procloraz, Procymidone, Chlorpyrifos, Fenitrothion, Linuron
Pharmaceuticals, Growth Promoters, and Personal Care Product Ingredients (section 3.1.1.7)	Endocrine active (e.g. Diethylstilbestrol, Ethinylestradiol, Tamoxifen, **Levonorgestrel),** Selective serotonin reuptake inhibitors (SSRIs; e.g. **Fluoxetine**), Flutamide, 4-Methylbenzylidene camphor, Octyl-methoxycinnamate, Parabens, **Cyclic methyl siloxanes** (D4, **D5,** D6), Galaxolide, 3-Benzylidene camphor
Other chemicals	
Metals and Organometallic Chemicals (section 3.1.1.8)	Arsenic, Cadmium, Lead, Mercury, **Methylmercury** Tributyltin, Triphenyltin
Natural Hormones (section 3.1.1.9)	17β-Estradiol, Estrone, Testosterone
Phytoestrogens (section 3.1.1.9)	Isoflavones (e.g. Genistein, Daidzein), Coumestans (e.g. Coumestrol), Mycotoxins (e.g. Zearalenone), Prenylflavonoids (e.g. 8-prenylnaringenin)

[1]See Appendix II for full names and abbreviations of the chemicals mentioned.

(e.g. phthalate esters), antimicrobials (e.g. triclosan), chemical stabilizing agents (e.g. phthalates), and metals (e.g. lead, arsenic, mercury). Most are added intentionally to these products but some may be contaminants with no added obvious benefit.

Pharmaceuticals for human or veterinary use contain EDCs and include contraception or other hormone therapies, lipid regulators, beta-blockers, anti-depressants, and antibiotics. Household, school and workplace products such as cleaners, toys, electronics, furniture, building materials, paints, paper, clothing, and lawn and garden supplies contain a range of chemicals including flame retardants (e.g. PBDEs), antimicrobial and chemical stabilizing agents, plasticizers, fragrances, solvents, preservatives, metals, and pesticides.

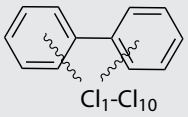
3.1.1.1 Persistent Organic Pollutants (POPs)

The majority of the POPs of the Stockholm Convention (http://chm.pops.int) are known as EDCs. They are listed as POPs as they have been shown to fulfill the criteria for persistency, bioaccumulation, toxicity and long-range transport as defined under the Stockholm Convention. In addition to these officially-acknowledged POPs, there are numerous chemicals that also fulfill some of the criteria of a POP. These are referred to in the table as "Other Persistent and Bioaccumulative Chemicals" (section 3.1.1.2, below).

These two groups of chemicals have high persistence and bioaccumulation potential, and are detected at elevated levels in wildlife and humans living close to where these chemicals were used or are still being applied, and in wildlife and humans far away from their sources. They also tend to be found at the highest concentrations in animals at the top of the food web (e.g. humans, seals, polar bears, birds of prey, crocodilians) and in tissues and body fluids that are high in fat (e.g. blubber, mothers' milk, egg yolk). Even though some of the POPs have been regulated or banned in most countries for several decades, such as the PCBs, many are still major global pollutants because of their persistence. This demonstrates the great challenge to eliminate highly persistent organohalogens from the environment and to prevent human and wildlife exposure. Furthermore, several types of POPs are composed of a number of congeners (homologues and isomers), making the total number of individual chemicals in commercial products and in the environment very high. For example, technical PCB products consist of about 130 congeners and PBDE-containing products can have up to 20-30 congeners. As a result, it is difficult to understand which specific PCBs or PBDEs are causing ED effects in wildlife and humans. Despite this, there is now a large body of evidence linking exposure to these chemical groups with endocrine diseases and disorders in both humans and wildlife (see Chapter 2, **Table 2.1** and sections 2.2 through 2.12).

Some POPs (e.g. PCBs, PBDEs) also undergo metabolism and have been shown to form ED active metabolites. These include PCB methyl sulfone and hydroxylated metabolites of PCBs (Letcher, Klasson-Wehler & Bergman, 2000) and PBDEs (Stapleton et al., 2009; Athanasiadou et al., 2008; Hakk & Letcher, 2003), even though some hydroxylated PBDEs are formed in processes other than internal metabolism (Ueno et al., 2008). Due to the persistent and bioaccumulative characteristics of the original (parent) molecules, there is a continuous internal source for formation of, e.g. hydroxylated PCBs and PBDEs. The PCB and DDE methyl sulfones, for example, are neutral compounds with high persistency and bioaccumulation potential (Letcher, Klasson-Wehler & Bergman, 2000). Intestinal microbial activity has been shown

Polychlorinated biphenyls (PCBs)



Characteristics: PCBs are technical mixtures of biphenyls with different numbers of chlorine atoms attached at different positions, making up a theoretical total number of 209 PCB congeners. PCBs exhibit high thermal and chemical stability and are very hydrophobic (log K_{OW} ranges from ~5.0 for Cl_2CBs to ≈ 8.9 for Cl_8CBs).

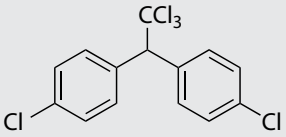
Origin and use: PCBs were produced from 1929 until the mid-1980s for primary use as insulating agents in transformer oils and capacitors, as heat transfer agents, and in sealants for construction (buildings).

Fate: PCBs are highly persistent in the environment, transported over long distances by air and water currents, and are globally distributed. As a result, wildlife and humans worldwide are exposed to PCBs. While some PCB congeners are easily metabolized, others are not. Some PCB congeners, particularly those with substitution at the 2,4 and 2,4,5 positions on the rings, accumulate through food webs to high concentrations in humans and wildlife.

Effects: Extensively studied: Possible endometriosis and fibroids in humans, fibroids, uterine tumours and adrenal problems in seals (Chapter 2.2 & 2.8). Strong experimental and molecular evidence for suppression of thyroid hormone in all vertebrate classes and epidemiological evidence of reduced cognitive function in children (Chapter 2.5 & 2.6). Limited evidence for increased prostate and breast cancer risk in humans and for genital carcinomas in sea lions (Chapter 2.7). Evidence for immune dysfunction in marine mammals and humans (Chapter 2.11). Limited evidence of increased diabetes risk (Chapter 2.10). Probable cause of population declines in fish-eating birds and mammals (Chapter 2.12).

Reviews: Hansen & Robertson, 2001; Ritter, Solomon & Forget, 1995; Waid; 1986; IPCS, 2003; 1992a; 1993

Dichlorodiphenyltrichloroethane (DDT)



Characteristics: Technical DDT is an organochlorine insecticide that consists mainly of 4,4'-DDT (structure shown) and 2,4'-DDT. DDT is very hydrophobic (log K_{OW} = 6.9). It is also semi-volatile and thus partitions into the atmosphere.

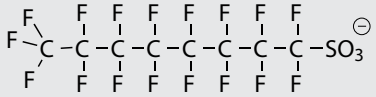
Origin and use: DDT was introduced during World War II, and has a broad range of agricultural and non-agricultural applications. Total global DDT production from the 1940s to present has been estimated at approx. 4.5 Mt (Li & Macdonald, 2005). Almost all uses of DDT were banned in the US, western Europe, Japan, and many other countries in the early 1970s, and in China and the former Soviet Union in the 1980s (Voldner & Li, 1993). The Stockholm Convention has given an exemption for the production and public health use of DDT for indoor application to control vector-borne diseases, mainly because of the absence of equally effective and efficient alternatives. However, both WHO and the United Nations Environment Programme, share a common commitment to the global goal of reducing and eventually eliminating the use of DDT without compromising the burden of vector-borne diseases (WHO, 2007a). In 2009, 13 countries in sub-Saharan Africa and 3 in southeast Asia used DDT to control malaria through indoor spraying (WHO, 2010a).

Fate: DDT and its related compounds are very persistent in the environment; as much as 50% can remain in the soil 10-15 years after application. DDT undergoes dehydrochlorination to DDE, which is a very persistent and bioaccumulative degradation product. In anaerobic sediments, dechlorination is the major degradation route, yielding DDD. DDT compounds are found globally in all environmental media due to their long-range atmospheric transport, great persistence and high bioaccumulation.

Effects: Extensively studied: Chapter 2, **Table 2.1**. Possible cause of endometriosis and disruption of ovarian cyclicity in humans. Eggshell thinning, feminization, homosexual behaviour and population declines in birds (Chapter 2.2 & 2.12). Lowered testosterone and demasculinization in polar bears and alligators and intersex in fish and frogs. Methylsulfonyl-DDE and o,p'-DDD cause adrenal hyperplasia and "Cushing disease-like" problems in seals (Chapter 2.8). Some evidence of suppression of thyroid hormone in marine mammals, birds and amphibia (Chapter 2.6). Limited evidence for increased breast cancer risk in humans (Chapter 2.7) and of leukemia and lymphoma. Limited evidence of increased obesity risk with perinatal exposure (Chapter 2.10). Probable cause of population declines in fish-eating birds and mammals (Chapter 2.12)

Reviews: Ritter, Solomon & Forget, 1995; Stemmler & Lammel 2009; WHO, 2007a; IPCS, 2011.

Perfluorooctanesulfonate (PFOS)



Characteristics: PFOS is a synthetic surfactant consisting of a perfluorinated C_8 chain and a terminal sulfonate group. Commercial mixtures contain both linear and branched isomers. PFOS is hydrophobic, oleophobic and proteinophilic (associated with proteins). It is exceptionally stable to degradation under natural conditions.

Origin and use: PFOS and the perfluorooctane sulfonyl fluoride (PFOSF) based products are produced by electrochemical fluorination. PFOS is commonly used as a salt or incorporated into larger polymers via amide or acrylate substituents. Production in the USA and Europe was phased out in 2001/02 but increased in China at that same time. PFOS-based polymers were incorporated into stain repellents and other surface coating agents. PFOS salts continue to be used in fire-fighting foams and in the semiconductor and photolithographic industry.

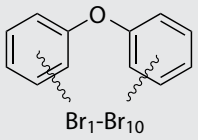
Fate: PFOS can be formed by environmental microbial degradation (or by metabolism in larger organisms) from PFOS-related precursors, i.e. molecules containing the PFOS-moiety. Due to the perfluorination, PFOS is highly resistant to any transformation. Volatile PFOS precursors, such as perfluorosulfonamides, are subject to atmospheric transport while long-range transport in oceans has been documented for PFOS. PFOS is bioaccumulative, but binds preferentially to proteins in liver and blood rather than accumulating in fats.

Effects: Little studied: Lowered female fecundity and altered menstrual cyclicity through occupational exposure. Reduced fetal growth (Chapter 2.2).

Reviews: Lindstrom, Strynar & Libelo, 2011; Stock, Muir & Mabury, 2010; UNEP, 2006

to be essential in formation of aryl methyl sulfone metabolites (Bakke, Bergman & Larsen, 1982; Bakke et al., 1983). A large number of PCB methyl sulfone metabolites are accumulated in humans and wildlife (Chu, 2003; Karasek et al., 2007; Letcher et al., 2009; Linderholm et al., 2007). The phenolic metabolites of POPs are covered in the group of halogenated phenolic compounds (section 3.1.1.5).

Polybrominated diphenyl ethers (PBDEs)



Characteristics: PBDEs are technical mixtures of diphenyl ethers with different numbers of bromine atoms attached at different positions, making up a theoretical total number of 209 PBDE congeners. PBDEs are brominated aromatic compounds of high chemical stability under natural conditions, but are broken down when heated. The PBDEs are hydrophobic (log K_{OW} of tetraBDEs = 6.77; heptaBDEs = 9.4).

Origin and use: PBDEs have been produced since the early 1970s for applications as flame retardants in textiles, electronics, electric articles, furniture and building materials. PBDEs have been subdivided into PentaBDE, OctaBDE and DecaBDE, representing the types of PBDEs produced commercially. DecaBDE (consisting predominantly of BDE-209) remains the major PBDE mixture in production worldwide with 85% of its global use occurring in North America and East Asia (BSEF, 2003).

Fate: PBDEs are very persistent in the environment, transported long distances by wind and air currents, and globally distributed. Debromination of DecaBDE - by sunlight in surface soils and on aerosols, and in the gastrointestinal tract of fish, mammals and birds - is a major transformation process that results in formation of less brominated BDEs (Schenker et al., 2008). Thus while "Penta" and "Octa" BDEs have been phased out, debromination of DecaBDE could be an additional source of emissions of the lower brominated congeners along with the large inventory of in-use PBDE products. While some PBDE congeners are easily metabolized, others are not. Some PBDE congeners bioaccumulate and biomagnify through food webs, and are present in wildlife and humans at high concentrations. Wildlife and humans worldwide are exposed to PBDEs.

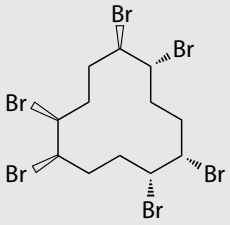
Effects: Limited evidence for earlier age at menarche and cryptorchidism in humans (Chapter 2.2 and 2.3), eggshell thinning, delayed hatching and reduced weight of hatchlings in birds (Chapter 2.2). Strong experimental evidence for suppression of thyroid hormone in humans and Arctic wildlife (Chapter 2.5 & 2.6). Limited evidence for cognitive disorders. Probable contributing cause of population declines in marine mammals (Chapter 2.12).

Reviews: Alcock, Mac Gillivray & Busby, 2011; Daso et al., 2010; EFSA, 2011a; Yogui, & Sericano, 2009; IPCS, 1994.

3.1.1.2 Other persistent and bioaccumulative chemicals

There are numerous other persistent and bioaccumulative organohalogens (chlorinated, fluorinated or brominated) besides those described above, including hexabromocyclododecane (HBCDD), short-chained chlorinated paraffins (SCCPs), hexachlorobutadiene, polychlorinated naphthalenes (PCNs) and pentachlorophenol which are under review for inclusion in the Stockholm Convention. Since HBCDD has been studied in quite some detail it is included as a case compound in the present chapter. However many other brominated flame retardants (BFRs) are not well or not at all studied with respect to their ED properties or environmental fate. For example, a number of BFRs, used as substitutes for PBDEs, have high persistence and bioaccumulation potential. Although there have been reviews of emerging BFRs (Covaci et al., 2011; Law et al., 2006; Eljarrat & Barceló, 2011; de Wit et al., 2011), very little exposure data have been published on any of these chemicals. Another large group of highly persistent chemicals are the perfluorocarboxylic acids (PFCAs). Perfluorooctanoic acid (PFOA) (see text box) is the most commonly assessed compound. Like PFOS (see section 3.1.1.1), the PFCAs all have a perfluorinated alkyl chain but with a carboxylate functional group.

Hexabromocyclododecane (HBCDD)



Characteristics: HBCDDs are cycloaliphatic compounds with six bromine atoms. Technically produced HBCDD is primarily a mixture of three stereoisomers, namely α-, β-, and γ-HBCDD, with enantiomeric pairs. γ-HBCDD is the dominant isomer in technical mixtures, with lower concentrations of α- and β-HBCDD. The HBCDDs have low water solubility and a high affinity for fats or organic carbon in soils and sediment. The γ-HBCDD isomer can be both abiotically and metabolically transformed to α-HBCDD. The dominant isomer in biota is α-HBCDD (see insert). Log K_{ow} (technical HBCDD): 6.6; (α-HBCDD): 7.9.

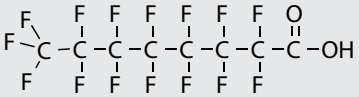
Origin and use: HBCDD has been produced from the early 1970s for use as a flame retardant in insulating materials in construction.

Fate: HBCDDs are transported long distances and generally present in wildlife and humans at concentrations in the low ng/g fat.

Effects: Little studied: Often found in association with PBDEs and PCBs in tissues and sometimes in association with the same effects.

Reviews: EFSA, 2011b; Law et al., 2005; 2008a; 2008b; Tanabe et al., 2008.

Perfluorooctanoic acid (PFOA)



Characteristics: PFOA is a fluorosurfactant consisting of a perfluorinated C_7 alkyl chain with a terminal carboxylate group.

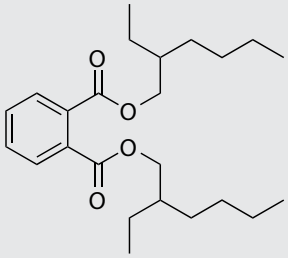
Origin and use: PFOA has been manufactured since the 1940s for industrial applications. The major application is as an emulsifier in the production of fluoropolymers (e.g. Teflon^R), but it is also used as an industrial surfactant in a variety of other processes. PFOA is also formed by the transformation of precursors such as polyfluorotelomers (including polymers incorporating the fluorotelomers) and by polyfluoroalkyl phosphates and phosphonates.

Fate: There are no indications of any transformation of PFOA in the environment. Neutral precursors of PFOA are subject to long-range atmospheric transport, and PFOA is transported long distances in ocean currents. The bioaccumulation potential of PFOA seems to be low in fish, but the presence of detectable concentrations in higher trophic levels (polar bear, caribou, walrus) has generated concerns regarding the biomagnification potential of PFOA in food webs. The voluntary PFOA Global Stewardship Program and the UNEP Strategic Approach to International Chemicals Management perfluoro initiative (both led by the US EPA) involve reductions of PFOA emissions and transitioning to alternatives.

Effects: Little studied to date but very limited evidence of in utero or perinatal exposure in association with adverse pregnancy outcomes in people (Chapter 2.2) and with obesity (Chapter 2.10). Adrenal glands are a potential target for these compounds (Chapter 2.8)

Reviews: Houde et al., 2011; Lindstrom, Strynar & Libelo, 2011; Stock, Muir & Mabury, 2010; Ahrens, 2011

Di(2-ethylhexyl) phthalate (DEHP)



Characteristics: DEHP is a diester, and an oily liquid at room temperature. It has low water solubility, and a log K_{OW} of around 7.5.

Origin and use: DEHP is widely used (>95%) as a plasticizer for polymers (mainly PVC but also other vinyl resins and cellulose ester plastics), and can be up to 30% of the product weight. The addition of DEHP improves the flexibility and workability of the plastics. Flexible PVC is used in medical devices, toys, cables, flooring, and other building materials. DEHP is also used as an additive in advanced ceramics for electronics and structural materials, and in printing inks, lacquers, paints, adhesives, sealants and rubber. In the EU DEHP is no longer permitted for use in toys and childcare articles and, in the USA, the Consumer Product Safety Improvement Act (2008) banned the use of six phthalates in toys and child care articles at concentrations greater than 0.1%.

Fate: DEHP has low reactivity under abiotic conditions except in the atmosphere. It is readily taken up by biota but undergoes metabolic transformations that are catalyzed by lipases and esterases in the gastrointestinal tract and primarily by lipases in other tissues. The monoester of DEHP is the primary metabolite of DEHP but several other metabolites are known.

Effects: Chapter 2, **Table 2.1** on DEHP and other phthalates. Limited epidemiological evidence of associations with fibroids and endometriosis in women. Extensive evidence for testis dysgenesis syndrome in experimental rodents; lowered testosterone, reduced anogenital distance, cryptorchidism, hypospadias and reduced semen quality. Limited evidence for associations between phthalate concentrations in mothers, urine and reduced anogenital distance, testosterone and sperm counts in their children. Very limited evidence for hyperactivity in girls exposed in utero (one study). Phthalate monoesters activate both rodent and human PPARα and PPARγ and also affect pancreatic beta cell function so may have strong negative impacts on human immune function (Chapter 2.11) and diabetes (Chapter 2.10). Limited studies show high plasma concentrations of DEHP are associated with endometriosis in women. Moreover, in accord with rodent studies showing effects on immunity, two case-control prevalence studies describe an association between the concentration of DEHP in indoor dust and asthma and wheezing in children.

Reviews: IARC, 2000; European Commission, 2008; Lyche et al., 2009.

There have been recent reviews of PFCs in the environment and biota (Houde et al., 2006; 2011). Other major classes of PFCs are perfluorinated alkylphosphonates and –phosphinates, and perfluorocyclohexane sulfonates (D'eon & Mabury, 2011; Lindstrom, Strynar & Libelo, 2011).

3.1.1.3 Plasticizers and other additives in materials and goods

The number of chemicals that can be included under this subheading is very large and they belong to very different classes of chemicals. Still, only a few classes of these chemicals have been investigated for their ED properties or toxicological profile in general. There is also an overlap in this section with several of the groups of chemicals described below such as halogenated and non-halogenated phenolic chemicals, pesticides, and additives in personal care products (sections

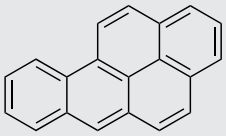
3.1.1.5, 3.1.1.6, 3.1.1.7), i.e. the individual chemicals can be listed in more than one group.

Plasticizers, e.g. phthalate esters (like DEHP, DBP, DiNP and BBP), adipic acid esters (e.g. DEHA) and non-pesticide organophosphate esters (e.g. triphenyl phosphate (TPP)), are commonly used additives in a number of materials and consumer products. Adipic acid esters and non-pesticide organophosphate esters are also used as additives for purposes other than as plasticizing materials. DEHP is used as an example of a phthalate ester plasticizer but it also has other applications (see text box). TPP is also used as a flame retardant additive in association with brominated flame retardants.

3.1.1.4 Polycyclic aromatic chemicals

Other ubiquitous persistent but non bioaccumulative compounds are polycyclic aromatic hydrocarbons (PAHs) that are generated during incomplete combustion of organic material (e.g. coal-fired power plants, residential heating, smoking) and they are also present in food (Boström et al., 2002; Fatoki, Ximba & Opeolu, 2011; Srogi, 2007). Several of the PAHs are referred to as EDCs, with the most well-known marker of PAHs being benzo[*a*] pyrene (BaP, see text box; e.g. Irigaray et al., 2007). PAHs are also commercial chemicals, some of which - e.g.-anthracene - are of very high concern (ECHA, 2012). Further, pyrene (four symmetrical fused rings) and anthracene (three rings) are both high production volume chemicals (US EPA, 2011b) that are used to make dyes and dye precursors.

Benzo[a]pyrene (BaP)



Characteristics: BaP is a polycyclic aromatic hydrocarbon. It is a pale yellow solid and has low water solubility (log K_{OW} = 6.1). BaP and other PAHs are classed as persistent organic pollutants under the United Nations Economic Commission for Europe POPs protocol.

Origin and use: The release of BaP to the environment is widespread as it is a product of incomplete combustion. BaP is mainly released from anthropogenic activities involving the combustion of fossil fuels and wood as well as from industries such as coke ovens and smelters. Major sources are transportation and domestic wood and coal combustion. Smoking of tobacco products or consumption of food, in particular barbecued food, constitutes an additional source of BaP.

Fate: Released BaP is largely associated with particulate matter, soils and sediment. In this form, BaP can undergo long-range atmospheric transport such that remote areas far from the source are exposed. In the gaseous phase, BaP can undergo rapid photooxidation.

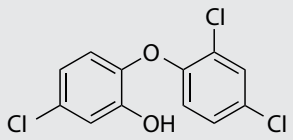
Effects: Exposure to PAH contaminated estuaries associated with neoplasia in wildlife but other contaminants also present (Chapter 2.7).

Reviews: IARC, 2010; Boström et al., 2002; Srogi, 2007

3.1.1.5 Halogenated and non-halogenated phenolic chemicals

Halogenated and non-halogenated phenolic chemicals (HPCs and non-HPCs) have emerged as EDCs of concern. The compounds can be of commercial origin, metabolites of POPs or other persistent organohalogens, or of natural origin. Halogenated phenols like PCP, 2,4,6-tribromophenol, triclosan and tetrabromobisphenol A are commercially produced for a variety of purposes, including use as pesticides (e.g. wood preservatives), antimicrobials and flame retardants. Many that persist are from the metabolism of POPs such as certain PCB (Letcher, Klasson-Wehler & Bergman, 2000) and PBDE (Athanasiadou et al., 2008; Hakk & Letcher, 2003) congeners and HCB (Koss et al., 1979). Phenols are also formed via demethylation of methoxyl groups in neutral compounds, such as pentachloroanisole and methoxychlor. HPCs are in many cases proteinophilic; as a result, they are found in blood and in proteineous tissues.

Triclosan



Characteristics: Triclosan is a trichlorinated phenoxyphenol with broad-spectrum antimicrobial action. It is relatively hydrophobic (log K_{OW} = 4.76). Methyl triclosan, a biotransformation product, is considered more persistent and bioaccumulative (log K_{OW} = 5.2).

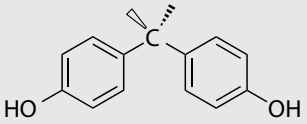
Origin and use: It is widely used in personal care products, but is also increasingly used in consumer products such as kitchen utensils, toys, bedding, socks, and trash bags.

Fate: Triclosan binds readily to soils and is not expected to evaporate from soil or water surfaces. In aquatic environments, triclosan attaches mainly to the surface of suspended solids and sediments and it also bioaccumulates in organisms. Effluents from sewage treatment works (STW) contribute to the widespread occurrence of triclosan in surface waters. Chlorination of STW effluents leads to formation of chlorinated triclosan products that are photochemically transformed to tri- and tetra-chlorinated dioxins when discharged into natural waters (Buth et al., 2011). Aerobic biodegradation is one of the major and most efficient biotransformation pathways for triclosan. Microbial methylation of triclosan has been reported, leading to the more lipophilic methyl triclosan with a higher bioaccumulation potential (Lindström et al., 2002).

Effects: Very little studied but disrupts steroidogogenic enzymes involved in the production of testosterone and estrogen. Could lead to reduced reproductive success in both males and females. Limited evidence of this in laboratory studies (Chapter 2.2 & 2.3). Limited epidemiological evidence for associations with hayfever and other allergies in humans (Chapter 2.11).

Reviews: Dann & Hontela, 2011

Bisphenol A (BPA)



Characteristics: BPA is an industrial chemical containing two 4-hydroxyphenyl rings. As a phenol it is moderately hydrophobic (log K_{OW} = 3.3) and water soluble (solubility = 120 mg/L).

Origin and use: BPA is a high-production volume industrial chemical used mainly in the production of polycarbonate plastic (~95%) and epoxy resins. Polycarbonate plastics are used in reusable food and drink containers including baby, milk and water bottles, in tableware, and in water pipes. Several countries have now banned the use of BPA based polycarbonates in baby bottles. The walls of cans and lids of glass jars and bottles for food and beverages are lined with epoxy resins as a protective coating. BPA is also found in some PVC plastic, and in a variety of paper products (e.g. thermal paper) and recycled paper. The European Food Safety Authority has set a tolerable daily intake (TDI) of 0.05 milligrams/kg body weight.

Fate: BPA is readily biodegradable and its potential to bioaccumulate is low. It can leach from food containers into solids and liquids and has been found in a variety of foodstuffs consumed by humans.

Effects: Environmental estrogen in all vertebrates. Limited evidence for prenatal exposure leading to disruption of estrus and premature cessation of cyclicity and fibroids in rodents (Chapter 2.2). Early exposure induces alterations in the mammary gland that render it more susceptible to neoplasia and to estradiol at puberty (Chapter 2.7). Acts via estrogen receptors in fat cells and cells of brain to regulate adipose tissue deposition and food intake in rodents. No human data. Affects beta cell function, increases insulin resistance and glucose intolerance and secretion in adult male rodents. Limited epidemiological evidence for link with type 2 diabetes and altered liver function in humans (Chapter 2.10). Reduction of thyroid hormone and metamorphosis in amphibians. Learning ability compromised in deer mice (one study), epidemiological evidence of increased maternal urine concentrations associated with increased defeminization of behaviour and hyperactivity (one study), consistent with rodent data, suggesting an effect of BPA on sexual dimorphism of these types of behaviours (several studies; Chapter 2.6).

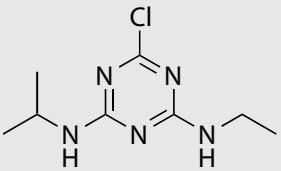
Reviews: WHO, 2011a; EFSA, 2010; WHO, 2011; 2010; Kang, Aasi & Katayama, 2007

Non-halogenated phenols include diethylstilbestrol (once an active pharmaceutical ingredient), bisphenol A, alkyl phenols and parabens. These chemicals have short half-lives but can, in the case of continuous elevated exposures, build up to concentrations of concern. Both HPCs and non-HPCs can also have a natural origin. For example, 2,4,6-tribromophenol and a hydroxylated PBDE (e.g. 6-OH-BDE47) are natural products, as are several phytoestrogens (see section 3.1.1.9).

3.1.1.6 Current-use pesticides

Increased public and regulatory attention during the 1970s and 1980s resulted in bans of pesticides, particularly those that are persistent and bioaccumulative, which led to the development and use of pesticides that are less bioaccumulative. Current-use pesticides are therefore generally characterized by shorter half-lives in the environment, with chemical properties that do not promote bioaccumulation in sediments or organisms. Still, some current-use pesticides are capable of building up to concentrations in soils and ground waters that may be of concern. These pesticides are used to control a wide range of pests and in a variety of applications. They can be used in building materials, during commercial or non-commercial crop production, by home owners for lawn and garden care, and for golf courses, and put into non-edible consumer products. The structural diversity among the almost 60 listed pesticides within the USA Endocrine Disrupting Screening Program (USA) is noteworthy (US EPA, 2009; 2010b), making predictions of relationships between chemical structure and ED activity very difficult. Many of these have been shown to be endocrine

Atrazine



Characteristics: Atrazine is a synthetic herbicide based on a triazine ring with chlorine and aminoalkyl substituents. Atrazine is relatively water soluble (33 mg/L) and chemically stable.

Origin and use: Atrazine has been widely used as a herbicide to control broadleaf and grassy weeds in major crops. The EU removed atrazine from its list of approved herbicides, but it is still used worldwide in many other countries. More than 35,000 tonnes were used annually in the USA alone in the mid-2000s.

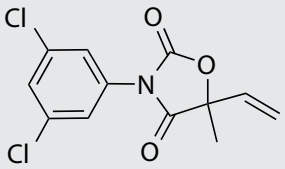
Fate: Atrazine is moderately to highly mobile in soils, especially where soils have low clay or organic matter content. It has a relatively long soil half-life, especially in cold climates. Because it is persistent and does not absorb strongly to soil particles, atrazine and its breakdown products have a high potential for surface and groundwater contamination. Atrazine is transformed to hydroxy compounds and via dealkylation in soils and surface waters.

Effects: Disruption of estrus in rodent studies (Chapter 2.2), intersex in frogs (Chapter 2.3). Exposure of rodents during prenatal period and during lactation results in depression of immune function in the the adult offspring. Amphibian and fish immune function is reduced by ecologically relevant concentrations, regularly accompanied by elevated infections (Chapter 2.11).

Reviews: Giddings et al., 2005; US EPA, 2006a

disruptors in vitro, albeit very few studies have been done in vivo, except for on a select few chemicals. Epidemiological evidence for effects of current-use pesticides and herbicides relates exposure to declines in male reproductive health (Chapter 2.3) and with breast cancer (Chapter 2.7). Several current-use pesticides have also been associated with prostate cancer (methyl bromide, chlorpyrifos, fonofos, coumaphos, phorate and permethrin) and with thyroid cancer (alachlor). In rodents, a number of pesticides induce thyroid follicular cell tumours, including amitrole, ethylene thiourea, mancozeb, acetochlor, clofentezine, fenbuconazole, fipronil, pendimethalin, pentachloronitrobenzene, prodiamine, pyrimethanil, and thiazopyr (Chapter 2.7). Finally, attention deficit disorder in children has been linked to elevated exposure to a variety of organophosphate pesticides (Chapter 2.6).

Vinclozolin



Characteristics: Vinclozolin is a dicarboximide fungicide. It is relatively hydrophobic (log K_{ow} = 3.1) and has a low vapour pressure (1.6x10^{-5} Pa).

Origin and use: Vinclozolin was used worldwide on oilseed crops, vines, fruits and vegetables. It was widely used in Europe until 2007 when a ban was implemented. The USA remains a major use area although uses on some crops were restricted in 2000 and current use is limited to canola and turf. These restrictions on use have led to reductions in quantities emitted to the environment.

Fate: Vinclozolin is transformed in plants and animals by cleavage of the oxazolidine ring to yield dichlorophenyl carbamate related metabolites. Vinclozolin is moderately persistent in agricultural soils with half-lives for total residues (vinclozolin plus its dichloroaniline-containing metabolites) of 179 to >1,000 days.

Effects: Concern about the toxicity of vinclozolin and/or its metabolites is related to its anti-androgenic activity and its ability to act as a competitive antagonist at the androgen receptor (EFSA 2008). Also its terminal metabolite, 3,5-dichloroaniline, is considered a potential carcinogen (US EPA 2000). See also Chapter 2.3, and **Table 2.1**. Anti-androgen that causes lowered testosterone, hypospadias, cryptorchidism, nipple development, and reduced penis size in male rodents. Feminization of male sexual behaviour and loss of sexual interest also in rabbits. Masculinization of females. Most sensitive period is during fetal development and transgenerational epigenetic effects have been reported (Chapter 2.3). Altered sex ratio in experimental studies with fish (Chapter 2.4). Reduced egg laying and fertility in birds (Chapter 2.2). Induces increased Leydig cell tumours in rats and so is a possible human carcinogen.

Reviews: EFSA, 2008; US EPA, 2000.

3.1.1.7 Pharmaceuticals, growth promoters, and personal care product additives

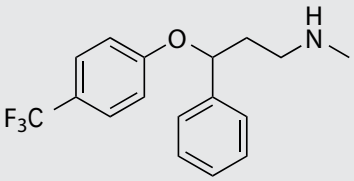
Endocrine-active pharmaceuticals have been developed over the years, and include chemicals to improve pregnancy outcomes (like diethylstilbestrol used to prevent miscarriages; see Chapter 2.1) and ones for contraceptives or hormone replacement therapies, such as synthetic estrogens (i.e. ethinylestradiol or EE2) and synthetic progestagen (e.g. levonorgestrel (Besse & Garric, 2009)) used in, for example, the birth control pill. With the exception of DES, which is no longer produced, many have been used for decades and are still on the market in a variety of formulations and products because of their effectiveness in birth control or hormone replacement therapies. The hormones present in pharmaceuticals, along with naturally-produced hormones, are excreted by women and men and not fully removed through the sewage treatment process. As a result, they are found in the effluents being discharged from sewage treatment works into receiving waters (Monteiro & Boxall, 2010). The feminizing and demasculinizing effects of estrogenic pharmaceuticals, in particular estradiol and ethinylestradiol, have been extensively studied in fish, both in the laboratory and in the field, where they cause intersex, and reduced fecundity and reproductive success in individuals (Chapter 2.3). Higher concentrations can cause population declines (Chapter 2.12).

Although there are many other pharmaceuticals on the market, the understanding of their potential effects on the endocrine system in wildlife, particularly in aquatic organisms, is currently very limited. However, some pharmaceuticals known to interfere with the endocrine system in wildlife include the selective serotonin reuptake inhibitor (SSRI) class of antidepressants. As for the oher endocrine-active pharmaceuticals, these chemicals are excreted by people using these pharmaceuticals and they enter the environment in complex mixtures mainly through the discharge of sewage or from the application of sewage sludge to soils (Monteiro & Boxall, 2010; WHO, 2011b).

Trenbolone acetate, a powerful anabolic steroid, is used in several countries as a growth promoter for beef cattle (Willingham, 2006). 17β-trenbolone is a relatively stable metabolic product of trenbolone acetate and enters the environment mainly in runoff from livestock feedlots. This metabolite has been shown to be strongly androgenic; for example, sex ratios of zebrafish exposed to trenbolone were skewed towards males (Morthorst, Holbech & Bjerregaard, 2010; Chapter 2.4).

Personal care products (shampoos, soaps, lotions, antiperspirants, cosmetics, toothpaste, etc.) contain a large number of chemicals, including some that are EDCs or suspected EDCs. Several of them have been mentioned above, such as parabens, triclosan, and phthalate esters. The cyclic methyl siloxanes are another class of chemicals used in personal care products. They are incorporated to reduce drying time in some rinse-off applications like shampoos and are used as carriers for aluminum salts in antiperspirants.

Fluoxetine



Characteristics: Fluoxetine is a secondary amine (making it ionizable), with two phenyl rings and limited water solubility (log K_{ow} = 4.05).

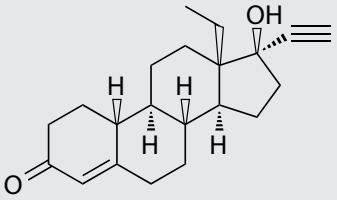
Origin and use: This is a selective serotonin reuptake inhibitor (SSRI) that is widely prescribed for depression, and for premenstrual dysphoric, panic, anxiety, obsessive-compulsive, and eating disorders.

Fate: This chemical is excreted mainly as metabolites by those taking the pharmaceuticals. Once in the sewage stream, fluoxetine and its metabolites may not be broken down during effluent treatment and can enter the environment with wastewater discharges.

Effects: Targets seratonergic modulation in the neuroendocrine brain and so has the potential to affect sex hormones, reproductive function and behaviour, feeding and energy metabolism in fish and other aquatic vertebrates (Chapter 2.6). Also influences the stress endocrine axis in fish (Chapter 2.8). Reduced growth and development, driven by reduced feeding rate in tadpoles (Chapter 2.6). Premature release of eggs and of non-viable larvae in freshwater molluscs (Chapter 2.6).

Review: Oakes et al., 2010

Levonorgestrel



Characteristics: Levonorgestrel is a steroid substituted with an ethinyl group. The compound is sparingly soluble in water, with a log K_{ow} of 3.

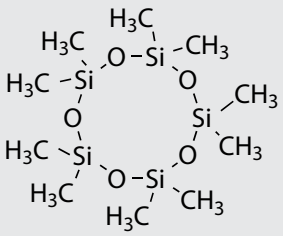
Origin and use: This is a progesterone-related chemical that is used in a number of oral contraceptives currently on the market.

Fate: This chemical is metabolized and excreted by those using levonorgestrel-containing pharmaceuticals. Once in the municipal wastewater stream, it can be found in the aquatic environment because it is not completely broken down during effluent treatment.

Effects: Little studied but detected at therapeutic levels in the blood of fish exposed to sewage effluents (higher than those in women on the contraceptive pill). Can cause reduced fecundity and cessation of egg laying in female fish and sub fertility and loss of sexual interest in male fish (Chapter 2.2 & 2.3).

Review: Besse & Garric, 2009

Cyclic methyl siloxanes



Characteristics: Cyclic methyl siloxanes, e.g. D4, D5 (the structure shown), and D6 are volatile, low-viscosity silicone fluids consisting of [-Si$(CH_3)_2$O-]× structural units in a cyclic configuration. They have an intermediate molecular weight (< 600 g/mol) and notable high vapour pressures (4.73-157 Pa). They are hydrophobic (log K_{ow} of 6.8 – 9.1).

Origin and use: D4, D5, and D6 are high production volume chemicals with a wide range of uses. They are used in hair and skin care products and antiperspirants and cosmetics (under the name cyclomethicone). The main use of D4 is for production of polydimethyl siloxane (PDMS, used for sealants and many other products) polymers and D4 is a residual (<0.1-3%) in these polymers. D5 and D6 are also used in production of PDMS. Volatile methyl siloxanes are used as an alternative to chlorofluorocarbons as a means of reducing the regulated volatile organic carbon content in products, and at parts per million levels as defoamers in pesticide formulations, pulp and paper, food, petrochemical, petroleum, chemical manufacturing, and water treatment. Thus there are direct releases to the atmosphere, to wastewaters and to indoor air.

Fate: D4, D5 and D6 are predicted to be persistent in air, with calculated atmospheric half-lives of more than 3 days. Thus they have the potential to be transported over longdistances in the atmosphere and have been detected in remote regions (Genauldi et al. 2011). Biodegradation studies indicate that D4, D5 and D6 undergo only limited transformation in most sediments or soils. However hydrolysis can be an important breakdown pathway in dry soils with high clay content (Xu, 1999; Xu & Chandra, 1999).

Effects: Little studied but some are estrogenic. Induce testicular atrophy and disturbances in spermatogenesis in several mammalian species (monkeys, dogs, rabbits and rats). Disruption of the estrous cycle and an increase in uterine weight in females (Chapter 2.2 & 2.3). No epidemiological studies.

Reviews: Environment Canada/Health Canada, 2008a; 2008b; 2008c. Genualdi et al., 2011.

3.1.1.8 Metals and organometallic chemicals

Many metals and metalloids are endocrine disruptors, disrupting a whole host of hormone pathways. They are found in rocks and soils and are common in ground and surface waters because of natural weathering processes. However, many metals are used in commercial products or are released into the environment during mining and metal smelting, the production of electricity using fossil fuels, and waste incineration. ED properties have so far been recognized for arsenic, cadmium, lead and mercury (mainly in its organic form methylmercury; see text box below).

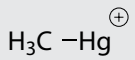
Arsenic is a metalloid found naturally in minerals worldwide and is present at trace levels in all soils, waters (ground and surface), and air (IARC, 2009). It is released to the atmosphere and to waters by weathering of rocks and volcanic eruptions. It is found in both organic and inorganic forms and its environmental fate and toxicity depend on the form of arsenic. High environmental levels of arsenic can be caused by combustion of fossil fuels, mining, and ore smelting. Similarly, arsenic has been used as a pesticide and in wood preservatives and these uses have led to localized contamination (IPCS, 2001).

Cadmium is a commonly occurring metal that is mainly found associated with ores of zinc, lead and copper. It is released from the earth's crust into water and air from natural weathering and from volcanic eruptions. Cadmium is also mined for a variety of uses including as protective plating on steel, stabilizers for PVC, pigments in plastics and glasses, and components of alloys and of electrode material in nickel-cadmium batteries. The mining, processing and disposal of cadmium-containing ores and products have resulted in high localized environmental contamination and this metal is also found as a contaminant in food (see reviews of cadmium by IPCS, 1992b; WHO, 2007b; IARC, 2009).

Lead is also a metal found commonly in rocks and soils worldwide, and is released through natural processes of geologic weathering and volcanic eruptions. Humans have used this metal in many different products including in batteries, ammunition, pigments for paints, ceramic glazes, crystal tableware and gasoline (as an additive), and it was put in solder used in food cans and water distribution pipes. A number of human activities can contaminate the air, water, and soils with lead including mining, smelting, burning of leaded gasoline or other fossil fuels, and recycling or disposal of lead-containing products (see also reviews of lead by WHO, 2007b; IPCS, 1995; 2012; IARC, 2006).

Metal exposure can target all five steroid receptor pathways (estrogen, progesterone, testosterone, corticosteroids and mineralocorticoids) and also receptors for retinoic acid, thyroid hormone and peroxisome proliferators (PPAR). It can affect embryonic, fetal and postnatal developmental processes in frog and fish models and in mice. Arsenic exposure, in particular, is strongly linked with increased prostate cancer risk (Chapter 2.7) and is also a powerful immunosuppressant (Chapter 2.11). Cadmium exposure has also been linked to prostate cancer in some, but not all epidemiological studies (Chapter 2.7). Some

Methylmercury



Characteristics: Methylmercury is an organic form of mercury that occurs naturally in the environment. It is proteinophilic, binding to the sulfhydryl groups in proteins.

Origin and use: Methylmercury is produced from inorganic mercury mainly by certain types of bacteria found aquatic systems. Inorganic mercury is present in rocks and soils and is released by weathering and by a number of human activities. The inorganic form of mercury has been used in a number of products (thermometers, fluorescent lights, batteries, switches, dental amalgams) and is released through natural (geological weathering, volcanic eruptions) and human (mining, chlor-alkali plants, coal and waste combustion) activities into the atmosphere or to terrestrial and aquatic systems. Methylmercury itself is not used in any consumer products or industrial processes but inorganic mercury is still used for mining and by industries in the production of various materials and goods.

Fate: The environmental cycling of methylmercury is complex and affected by many different physical, chemical or biological processes (**Figure 3.5**). Methylmercury has a high affinity for particles or dissolved organic carbon in waters. It also concentrates into bacteria, plants and algae at the bottom of aquatic food webs and then is transferred and biomagnified as those organisms are eaten by progressively higher levels in the food web. The biomagnification of methylmercury in aquatic food webs can lead to high concentrations in fish-eating fish, birds, and mammals (including humans). Many countries have fish consumption advisories to reduce the risk to human consumers, and there is a current global review of mercury led by UNEP to be finalized in 2013 (UNEP's Global Mercury Partnership (UNEP 2011a)). Human activities have increased the levels of mercury in the freshwater and terrestrial environment by 2-3 times.

Effects: Well studied element. Mercury crosses the blood-brain barrier and reduces the levels of key enzymes critical for reproduction, cognition, growth and development in vertebrate wildlife. Elevated exposure of fish and amphibians to methylmercury also impairs behaviours that are critical for successful reproduction, avoidance of predators and feeding; younger animals are more sensitive. In aquatic birds methylmercury exposure at environmentally relevant levels can interfere with reproductive success due to neuroendocrine disruptive effects on courtship behaviour and mate choice (Chapter 2.6). These effects may be relevant to the sustainability of bird populations (Chapter 2.12).

Reviews: Pacyna et al., 2010; WHO, 2010b; Dietz, Outridge & Hobson, 2009; Tan, Meiller & Mahaffey, 2009; Mergler et al., 2007; WHO, 2007b; 2007c; IPCS, 1990.

metals, such as cadmium and arsenic, also have diabetogenic effects in rodents and for arsenic, similar effects are indicated in epidemiological studies (Chapter 2.10). Lead has been particularly associated with delayed puberty (Chapter 2.2) and with cognitive problems in children, and incuding attention deficit disorder (Chapter 2.6).

Organotin compounds [e.g. tributyltin (TBT)] are also of concern due to their ED activity. TBT was heavily used in anti-fouling paints for boats and ships, but is now mainly banned from use because of its persistence, and its accumulation in and effects on aquatic wildlife, particularly snails, living in areas with heavy boat traffic (see Chapter 2.2, 2.4, 2.10-2.12). Tributyltin compounds (oxides, chlorides, hydrides) continue to be used in wood preservatives and as chemical intermediates (ECHA, 2011; US EPA, 2011b). Triphenyltin hydroxide/ acetate, once widely used as a fungicide (IPCS, 1999), has been restricted or banned for most agricultural uses in Europe and the USA However, other triphenyltins remain in commerce (ECHA, 2011; US EPA, 1999b; 2011b).

3.1.1.9 Natural hormones and phytoestrogens

Humans and mammals excrete the steroid hormones, e.g. estradiol, estrone, testosterone and estriol, used for controlling sexual development and reproduction. Considerable amounts of natural steroidal hormones are also produced by livestock and found in animal wastes. Some of these natural hormones are released directly to the environment in untreated sewage, or are not completely broken down during wastewater treatment and discharged in sewage treatment works effluents. In addition, animal wastes from agriculture can be a source of hormones to the terrestrial and aquatic environments when manure is applied to fields and the hormones are washed with rainfall into nearby streams. These endogenous steroidal EDCs are characterized by extremely high potency as compared to other non-hormone EDCs (for effects on fish see Chapter 2.3).

Phytoestrogens are plant-derived natural non-steroidal compounds that show estrogenic activity due to their structural similarity with the female sex hormones. Some typical phytoestrogens are the isoflavonoids, coumestans and prenylated flavonoids. A Canadian study on the levels of nine common phytoestrogens in a Western diet showed that foods with the highest relative phytoestrogen content were nuts and oilseeds, followed by soy products, cereals and breads, and legumes (Thompson et al., 2006; Pongratz & Vikström Bergander, 2011). The natural steroidal hormones and phytoestrogens are not included further in this chapter since the focus is on anthropogenic EDCs. However, it is important to recognize that these chemicals are also contributing to the overall issue of EDCs (see Chapter 2.2).

3.1.2 Structural features of EDCs

In general it is not possible to determine whether a chemical is an endocrine disruptor based on its structure, as there are a multitude of mechanisms and pathways by which a chemical can have an effect. In some cases, endocrine activity may not be attributed to the parent compound, but instead to one or several of its metabolites. Still there are some structural features that are indicative of estrogenic, thyroidogenic and glucocorticoid activities. Estrogenic compounds often include a phenolic ring, similar to endogenous (naturally-produced) estrogens, and no halogens, as exemplified in **Figure 3.1**. The figure also shows a few estrogenic compounds with very different structures. Typical thyroidogenic EDCs are substituted with chlorine or bromine atoms next to the OH group in a phenol and are similar in structure to natural thyroxin (T4), as exemplified in **Figure 3.2**. The aryl methyl sulfones shown in **Figure 3.3** indicate the importance of the sulfone functional group for interference with glucocorticoid hormone activity.

3.1.3 Environmental distribution and fate of EDCs

EDCs enter the environment during their production, use and disposal (**Figure 3.4**). When raw chemicals are manufactured, EDCs are released in industrial discharges. However, larger emissions occur when chemicals are incorporated into materials and products - such as plastics, furniture, carpets, and electrical equipment - by downstream manufacturers and when these goods are used. Emissions during manufacture or use can be to water, soils, and the atmosphere, due to the release of EDCs from materials and products, and from the disposal, incineration or recycling of wastes (e.g. e-wastes). The chemicals in products used in homes for cleaning, bathing, and health are washed down the drain or flushed down the toilet into the sewage system. Some EDC-containing sewage effluents enter the environment untreated, while others receive some form of treatment before they are released to aquatic systems. Other sources of EDCs to the environment include runoff from agricultural fields, emissions from urban areas, and long-range transport via air and ocean currents to remote environments. EDCs enter the environment from both point (discrete) and non-point (diffuse) sources as described below. There are no environmental distribution and fate characteristics that are specific to EDCs because of their broad range of physical and chemical properties.

3.1.3.1 Point sources

Sewage effluents have been identified as a source of a diverse mixture of EDCs to the aquatic environment. These waters from homes and industries include natural and synthetic hormones (estrogens, androgens), active ingredients in pharmaceuticals, metals, pesticides, personal care product additives, and industrial chemicals. Over one hundred pharmaceuticals (not including their metabolites) used by humans have been detected in effluents and surface waters at concentrations ranging from low parts per trillion (ng/L) to parts per billion (µg/L) and include analgesics, anti-inflammatories, anti-depressants, anti-epileptics, lipid regulators, several classes of antibiotics, β-blockers, antineoplastics, and hormones (Monteiro & Boxall, 2010).

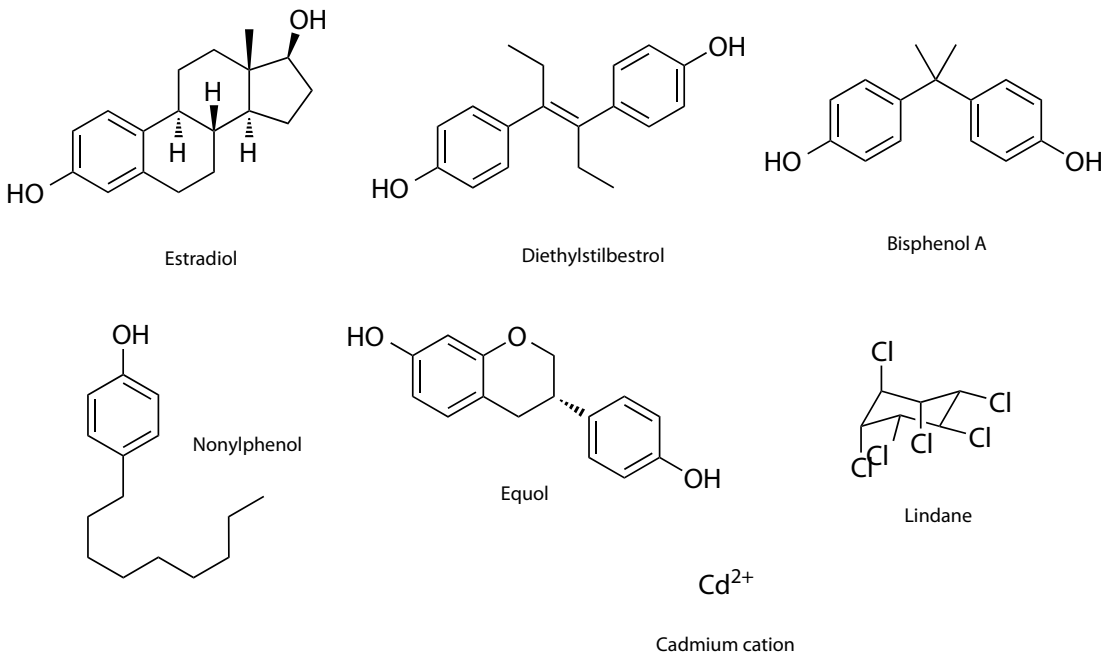


Figure 3.1. Chemical structures of a few estrogenic compounds, including natural 17β-estradiol. The examples show the great variability in the structures of chemicals that have estrogenic effects.

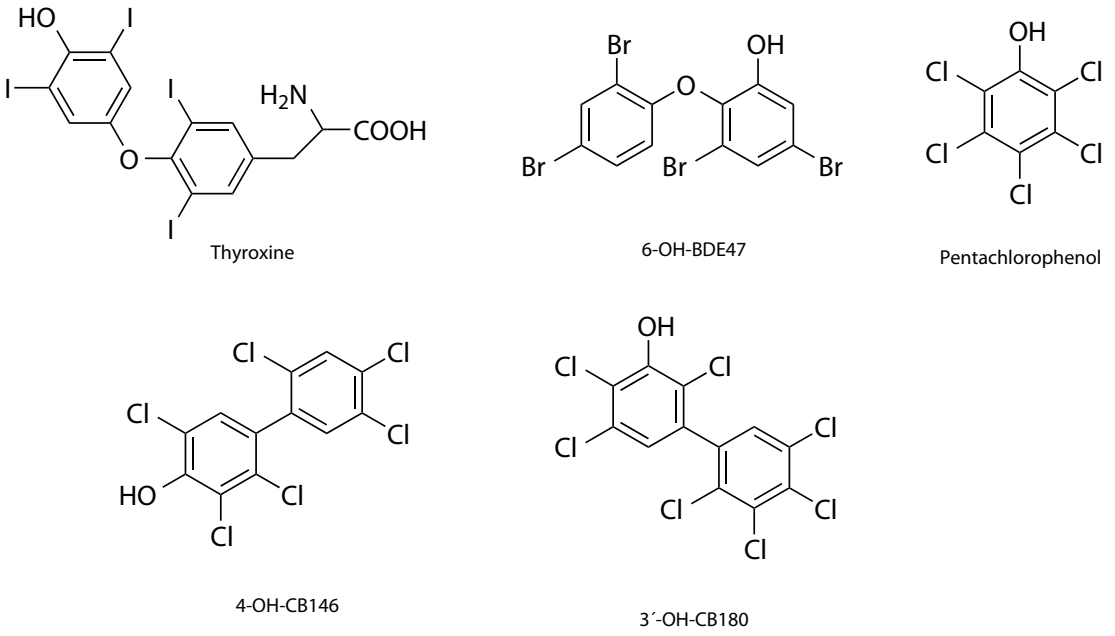


Figure 3.2. Chemical structures of a few thyroidogenic compounds, including natural thyroxin (T4). There is, at least in part, a general structural resemblance between the chemicals with effects on the thyroid system. Abbreviated names are given in full in Appendix II (List of chemicals).

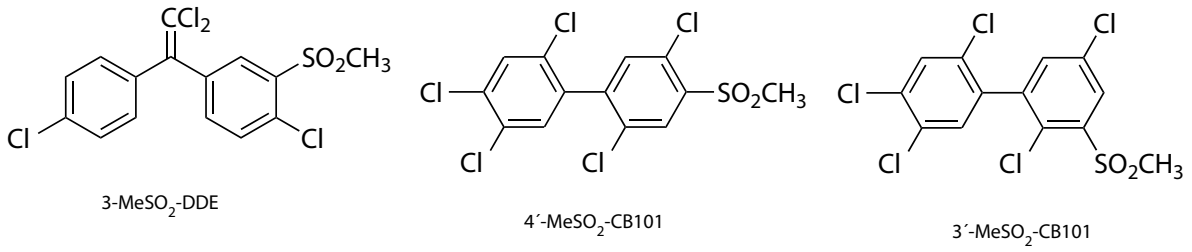


Figure 3.3. Chemical structures of aryl methyl sulfone compounds, all known to exhibit glucocorticoid activity. Abbreviated names are given in full in Apppendix II (List of chemicals).

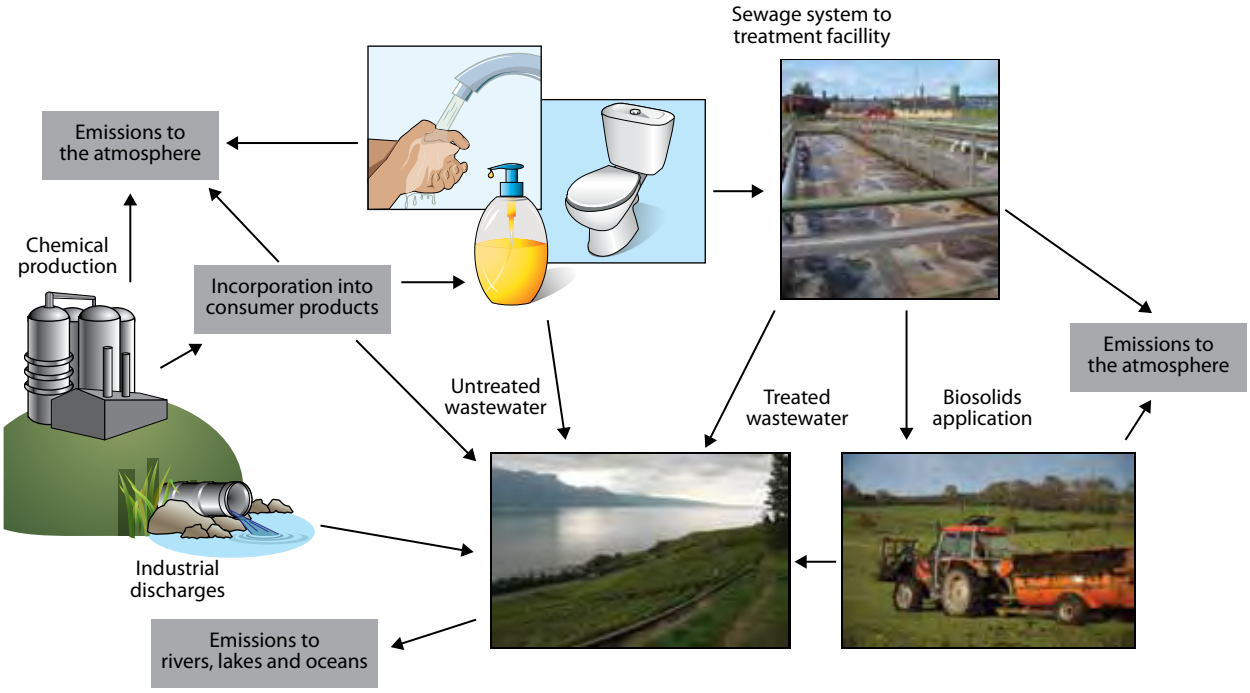


Figure 3.4. Environmental releases of EDCs from the manufacturing stage through use and disposal of personal care products and pharmaceuticals.

Some human pharmaceuticals are excreted in urine and faeces as metabolites (e.g. carbamazepine, an antiepileptic drug, has 5 different metabolites) and these compounds can be found in wastewaters at much higher levels than the parent chemical (la Farré et al., 2008). The kinds and concentrations of chemicals found in the effluents and receiving waters depend on the source of the wastewaters (industrial, municipal (this also depends on population demographics)) and the type of treatment the wastes received. Other point sources of concern include industries producing EDCs for commercial use; for example, effluents from pharmaceutical companies have been found to contain high concentrations of ciprofloxacin (Fick et al., 2009; Larsson, de Pedro & Paxeus, 2007). Globally, however, municipal wastewaters are believed to be a much greater source of pharmaceuticals to the environment (Monteiro & Boxall, 2010; WHO, 2011). The following are some examples of EDCs present in sewage and other point source discharges.

Triclosan: Sewage treatment works vary widely in their ability to degrade the antimicrobial triclosan. As a result, it is commonly found in municipal effluents and biosolids up to 3 µg/L and 33 µg/g, respectively, and is also detected in receiving waters (< 2.3 µg/L) and sediments (< 800 ng/g) (see review by Dann & Hontela, 2011). During the wastewater treatment process, triclosan is also converted via biomethylation into methyl triclosan, a chemical that is more persistent in the environment and more likely to accumulate in fish.

PFOS: Another EDC commonly found in sewage effluents and waters downstream of the discharges is PFOS. This chemical has been detected in effluents (up to 993 µg/L) and river waters (up to 193 µg/L) (see review by la Farré et al., 2008).

Bisphenol A: This chemical is present in municipal and industrial (producing chemicals and chemical products, printing paper, paper recycling, and packing-board paper plants) wastewaters. It is not completely broken down during wastewater treatment (37-94%) and is present in the effluent and sludge (Kang, Aasi & Katayama, 2007; Lee & Peart, 2000). As a result, it has been detected in ground and surface waters (up to 20 µg/L), in river sediments (up to 1.63 mg/kg dry weight), and in the leachate from landfills (Focazio et al., 2008; Kang, Aasi & Katayama, 2007).

Fluoxetine: Due to the variable effectiveness of sewage treatment in removing fluoxetine (ranges from <40 to >90%; Monteiro & Boxall, 2010), this antidepressant has been measured in municipal wastewater effluents and downstream waters at a number of locations worldwide. Concentrations up to 99 ng/L, 46 ng/L and 0.37 mg/kg dry weight have been reported in effluents, river waters, and treated sewage sludge, respectively (Monteiro & Boxall, 2010).

Levonorgestrel: Few environmental measures of this progestagen exist. However, some studies have measured it at low ng/L concentrations in municipal effluents (e.g. Fick et al., 2010).

The EDCs present in sewage occur as either the parent compound or as metabolites. Microbial transformations during sewage treatment can convert metabolized chemicals back to the parent compounds, resulting in the release of EDC-active chemicals; for example, conjugated ethinylestradiol excreted by women taking the birth control pill is converted back to the original parent form in sewage treatment works. Demethylation is known to play an important role in the transformation of phytoestrogens and methoxychlor to ED-active species (Cravedi & Zalko, 2012).

3.1.3.2 Non-point sources

In areas of food production, storm runoff from agricultural fields is an important non-point source of EDCs (pesticides, hormones, pharmaceuticals) to aquatic systems. The pesticides used on crops and for other agricultural purposes are found in ground water and in streams in these regions, especially after a heavy rainfall. Animal wastes are also washed into surface waters by rainfall and contain endogenous hormones, growth promoters and pharmaceuticals. Other EDCs are found in the sludge (biosolids) that remains after sewage is treated (Citulski & Farahbakhsh, 2010). If these solids are applied to fields, EDCs like triclosan are found in nearby surface waters (Edwards et al., 2009).

Urban areas have high rates of material, energy and chemical use, leading to emissions of a diversity of chemicals into the environment (Hodge & Diamond, 2010). Typical EDCs emitted in urban areas are: PAHs from fossil fuel combustion by vehicles; PCBs from older paints and sealants; BFRs from consumer goods with flame retardants; and PCDD/Fs from medical or municipal waste incinerators. Emissions are difficult to quantify as they originate from a myriad of sources, but clear, decreasing chemical concentration gradients have been found from urban to rural locations (Harrad & Hunter, 2006).

Some EDCs are transported long distances to remote environments via air and water currents. The atmospheric pathways are particularly important for highly persistent, semi-volatile EDCs such as PCBs, DDTs, pentaBDEs, current-use pesticides (e.g. endosulfan), as well as PFOS precursors (perfluorosulfonamido alcohols) and PFCA precursors (polyfluorotelomers). Highest concentrations of HCH isomers in the world's oceans are in the Arctic, great distances from where they were originally released (deWit et al., 2004). Highly persistent water soluble chemicals (e.g. PFOS and PFOA, see below) are thought to be transported to remote environments mainly via ocean currents (Armitage, MacLeod & Cousins, 2009; Armitage et al., 2009; Prevedouros et al., 2006) although atmospheric transformation of their volatile precursors also contributes to the burden in remote environments (Schenker et al., 2008; Wallington et al., 2006). The β-HCH isomer is also thought to be transported mainly via ocean currents. β-HCH is the predominant chemical form of HCH of concern for top aquatic predators because it biomagnifies and apparently resists transformation (Willett, Ulrich & Hites, 1998).

Non-point sources are also important for contamination of remote environments with PFCs. Transport by ocean currents is thought to be the main pathway for the global distribution of PFOS and PFOA (Armitage, Mac Leod & Cousins, 2009; Armitage et al., 2009; Stemmler & Lammel, 2010; Wania, 2007; Yamashita et al., 2008). Redistribution of these contaminants from lower latitudes to the Arctic Ocean

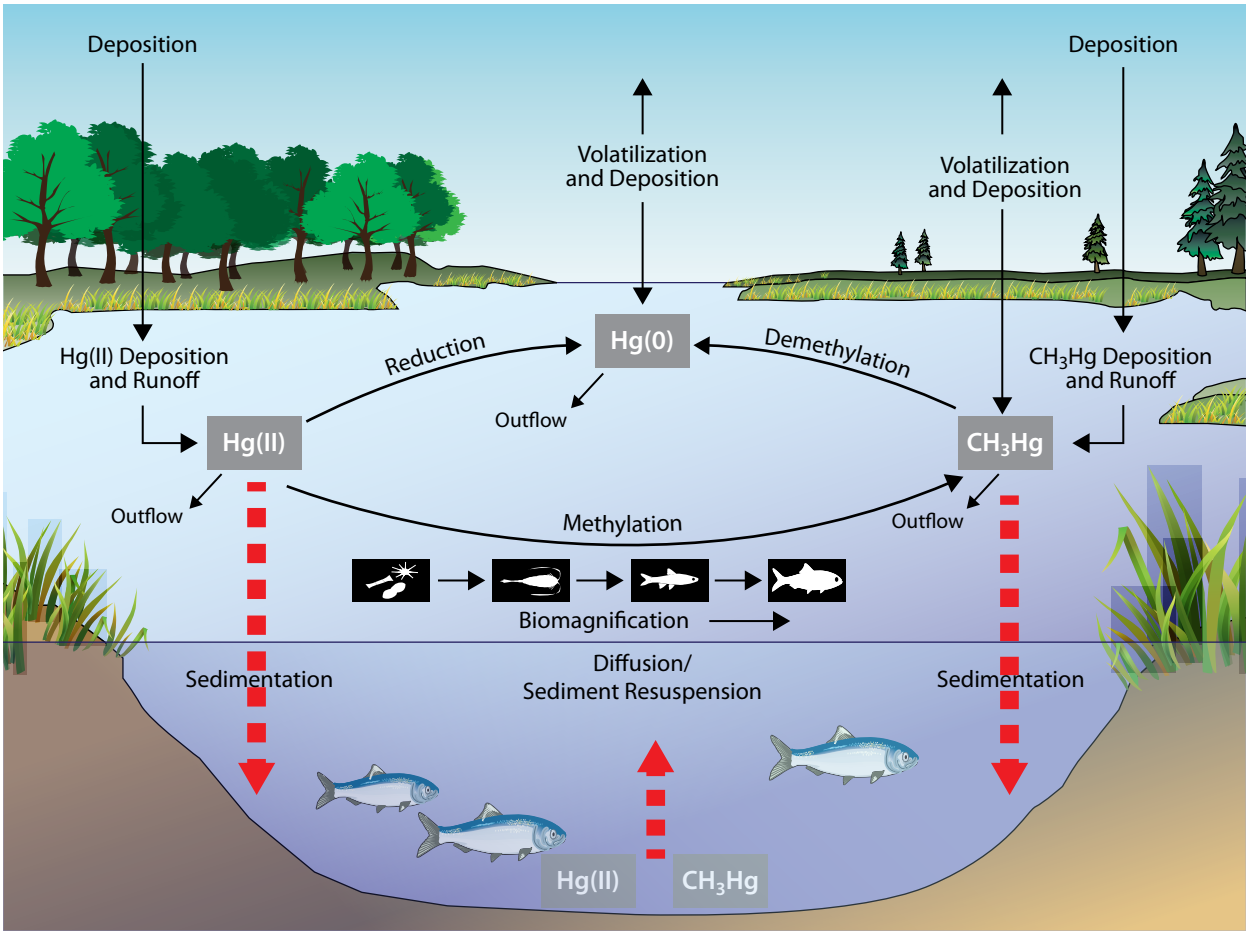


Figure 3.5. The complex cycling of mercury and methylmercury in the aquatic environment (modified from Watras & Huckabee, 1994).

is ongoing and the total mass (and average concentration) of PFOA and PFOS in the marine environment is expected to increase for the next 10 to 20 years based on modeled predictions (Armitage, Mac Leod & Cousins, 2009; Armitage et al., 2009; Wania, 2007). However, the withdrawal of PFOS from commerce in North America and Europe, as well as its inclusion in the Stockholm Convention, should lead to declining concentrations in the environment. On the other hand, there are many chemical precursors of PFOA that continue to be produced, resulting in continued emissions to the atmosphere as well as to surface waters (Armitage, Mac Leod & Cousins, 2009). Loss through settling and mixing to the deep waters of the ocean was estimated to remove approximately 25% of the total global emissions of PFOA over the period 1950 to 2004 (Armitage et al., 2009), which implies that these undegradable PFCs will be circulating in the world's oceans for many centuries after emissions to surface waters cease.

Mercury is an EDC that has very important non-point source emissions due to its release to the atmosphere from both natural sources (e.g. geological weathering) and human activities (e.g. burning of fossil fuels and wastes) (Pacyna & Pacyna, 2001; Pacyna et al., 2010). The mercury cycle is very complex and affected by a number of abiotic and biotic processes (**Figure 3.5**). Gaseous elemental mercury, one of the forms released to the air, has an atmospheric half-life estimated at between 6 months and 2 years (Lin et al., 2006; Strode et al., 2008) and can therefore undergo long-range transport and deposition (AMAP/UNEP, 2008). Rainfall removes some forms of mercury from the atmosphere. Once deposited, these mercury species can be chemically reduced to gaseous elemental mercury and then reemitted to the atmosphere (Steen et al., 2009). The repetition of the deposition and reemission cycle constitutes mercury's so-called "grasshopper" motion (Almeida et al., 2005). Thus, mercury that is emitted in one part of the world can eventually be transported to any other location. Mercury deposition in remote freshwater environments has increased two, to three-fold since the advent of the Industrial Revolution (Fitzgerald et al., 2005; Muir et al., 2009). For fish and wildlife the most important transformation is the conversion to methylmercury (**Figure 3.5**), because this is the form that biomagnifies through aquatic food webs and has ED effects in wildlife and humans (WHO, 2010b; 2007b; IPCS 1990; Chapter 2.6).

3.1.4 External exposure of wildlife to EDCs

Understanding the exposure of wildlife to EDCs is crucial but very challenging because some EDCs are not persistent in the environment or organism, and there are a large number of chemicals present in their food and habitat. Wildlife are not exposed to individual chemicals in isolation but to mixtures of chemicals that can affect the endocrine system in similar or opposite ways. It is clear that the timing and levels of exposure are critical to understand because some developmental stages for wildlife, e.g. during the fetal period (Hamlin & Guillette, 2011), are much more sensitive to EDCs than others. Although EDCs can be present in the environment, exposure must occur for the chemical to have an effect. While there is a better understanding of what is present in the abiotic environment, information on what is transferred into organisms – especially to target organs – is often incomplete or missing. In the absence of measured data, environmental fate and bioaccumulation models are generally used to predict exposures of wildlife to EDCs in regulatory risk assessments. Attributing endocrine effects to specific chemicals or groups of chemicals and understanding critical exposures remains a challenge and a research priority.

The exposure of wildlife to EDCs in the environment will come from air, water, food, soil or sediment and depends on the properties and persistence of the EDC. While some are rapidly transformed by sunlight, bacteria and chemical processes, others resist breakdown and can remain in the environment for months to years. As a result, exposures will vary considerably from one type of EDC to the next and it is not possible to define a "typical" route, level, or duration of exposure for the diverse group of substances that are known to be EDCs. Organisms can take up these chemicals from their diet, by inhalation or by absorbing them through the skin, where they travel in the blood to specific tissues and affect the endocrine system.

Wildlife exposures to EDCs are assessed through measures of their external environment (air, water, soil, sediments, food) and also by analysing the chemicals present in their tissues (see section 3.2.1.2). **Figure 3.6** shows the routes of exposure for fish (a representative aquatic organism) and polar bears (a representative marine top predator) to EDCs. Polar bears are typically not exposed to local sources; they have been shown to do little feeding while on land or near dumpsites in communities in the Arctic (Ramsay & Hobson, 1991). Yet as top predators they have some of the highest levels of PFOS, PCBs and organochlorine pesticides of any species due to the long-range transport, deposition and food web biomagnification of these chemicals.

While exposures of species living in remote locations, like the polar bear, are important to understand, aquatic and terrestrial species living in or near urban areas are often continuously exposed to EDCs via sewage treatment works outfalls, urban and agricultural runoff, and industrial effluents (**Figure 3.7**). Once effluents are discharged to aquatic environments, EDCs will be diluted in stream or river waters so that organisms living very close to the discharge will have the highest exposure. Although some EDCs are not persistent in waters (e.g. estrogens persist days to weeks), there is a constant discharge and fish and other organisms are continuously exposed to a mix of persistent and "pseudo persistent" chemicals. Additional exposure sources, especially in marine environments, are accidental or intentional discharges from oil tankers, ships and fuel extraction activities and oil spills.

Water exposure - The importance of water as a source of EDC exposure for wildlife depends on the type of chemical. Some EDCs are more soluble in water (e.g. some pharmaceuticals, current-use pesticides, natural hormones such as estrogens), are found at parts per trillion (ppt, ng/L) to parts per billion (µg/L) levels downstream of their sources, and water

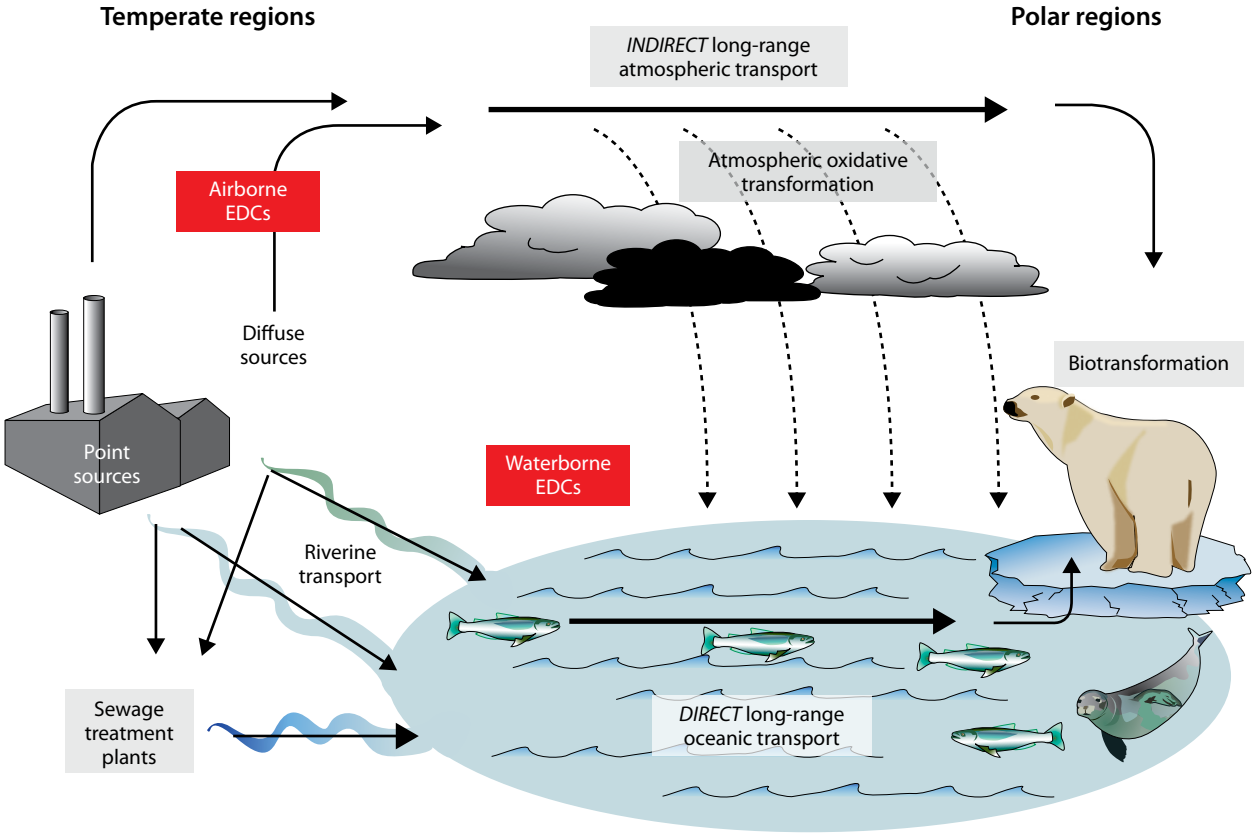


Figure 3.6. Routes of EDC exposure for biota in remote environments, illustrating the importance of long-range transport pathways for wildlife. Based on AMAP (2009).

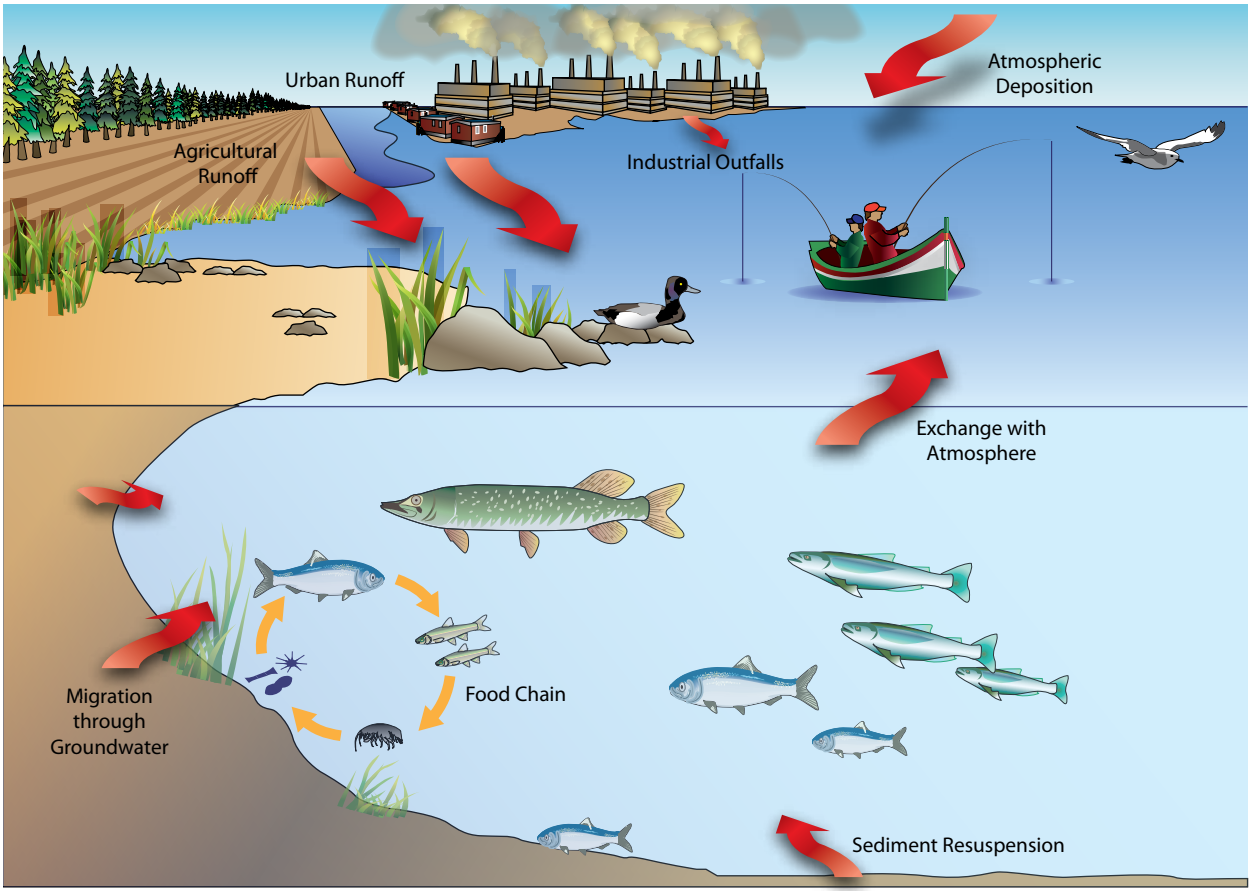


Figure 3.7. Exposure of fish and wildlife in urban regions due to continuous release of EDCs in effluents and to the atmosphere (Redrawn based on a figure from Chapter 4 of The Great Lakes: An Environmental Atlas and Resource Book, www.epa.gov/greatlakes/atlas/).

is the main route of exposure for wildlife to these chemicals. Fish will take these EDCs up through their gills, whereas birds and mammals will be exposed primarily through their drinking water. A diverse mixture of chemicals is present in surface waters and concentrations vary from one site to another and over time at the same site (e.g. Focazio et al., 2008).

Sediment or Soil Exposure- When EDCs are released into the environment, some will bind to soils or to particles and sediments in rivers or other waterways. Organisms living in or on the soils or sediments (worms, snails, some insects) are exposed to these particle-bound EDCs and the EDCs can concentrate into these organisms and up the terrestrial or aquatic food web. While there is a good understanding of how some of the POPs (e.g. PCBs) can, and do, bind to sediments and soils, much less is known about the environmental fate of chemicals of more recent concern. Very few measurements of sediment-bound pharmaceuticals, for example, have been made downstream of sewage treatment works discharges, and it is not well known whether there is much or any exposure of organisms to EDCs through this route.

Diet exposure- Diet is an important source of EDC exposure for wildlife. A number of EDCs are POPs with high affinities for fats and low solubility in water. Because of these properties, these chemicals are well known to concentrate in organisms and through food webs, with higher levels in fish-eating species than those that feed lower on the food web. This concentration – biomagnification – has been well described for many of the chlorinated pesticides (DDT, chlordane, toxaphene) and other POPs (PCBs, PBDEs, HBCDD), as well as for some metals (methylmercury) of concern to the endocrine system. The concentration of EDCs an organism is exposed to depends on the type of diet it consumes, as diets high in fats will have the highest levels of fat-soluble EDCs like POPs. In addition, because these chemicals are taken up into the body faster than they are lost, there is an accumulation of many of them as an organism grows and ages such that higher levels of POPs and methylmercury are found in the older, bigger animals. These processes are well understood for the POPs, but are still poorly understood for the EDCs that are only more recently being measured in the environment (e.g. triclosan, active ingredients of pharmaceuticals).

3.1.5 External exposure of humans to EDCs

The major exposure pathways for humans to many EDCs are via food and drinking water. However, over the past decade it has become clear that humans, in particular small children, are also exposed to EDCs via dust and particles in indoor environments like homes, schools, childcare centres, and offices. These EDCs are released from materials and goods in homes and at work; they are additives in electronics and electric products, textiles and furniture. Also handling of waste (e.g. e-waste) and recycling have been identified as sources of external exposure to EDCs for humans (see section 3.1.5.1). Humans can take up EDCs, or precursors of EDCs, through inhalation of air and particles, consumption of contaminated food and drinking water, and through direct dermal contact, e.g. with cosmetic products (**Figure 3.8**). Human exposure varies considerably and depends on individual habits (e.g. food choices), and the locations where people work and live.

For most adults, exposure to POPs and other persistent organohalogens is primarily through the consumption of fatty foods. However, persistent and bioaccumulative compounds such as BFRs and PFCs are still in use in a great variety of consumer products, and recent studies have demonstrated that concentrations are much higher in indoor than in outdoor environments (e.g. Harrad et al., 2010). It has become clear that infants and small children are at particular risk for exposure to these chemicals through their high hand-to-mouth activity, i.e. ingestion of contaminated dust/particulate matter (Haug et al., 2011; Goosey & Harrad, 2011; Lunder et al., 2010; Trudel et al., 2011). Adults can be exposed to POPs via inhalation but this is most commonly related to occupational exposure. For example, median concentrations of DDT, in blood of spray operators were 11 times higher than those of people living in homes that were sprayed (Table 1 of IPCS, 2011). Dermal uptake of POPs and other persistent and bioaccumulative compounds is regarded as a minor pathway for non-occupationally exposed people.

Humans can be exposed in several ways to other EDCs in materials and goods (e.g. triphenyl phosphate, phthalates, bisphenol A); for example, bisphenol A is in the lining of some food containers and in some paper products such as cash register receipts (Liao & Kannan, 2011; Muncke, 2009). A large number of chemicals are used as additives in indoor materials, food packaging, and other consumer products, and these compounds can leak from the packaging, materials and goods into food or onto dust that is ingested primarily by toddlers and infants. Skin uptake can be particularly relevant for chemicals used in, e.g. cosmetics and other personal care products, but can also occur when receipts are handled (Geens, Goeyens & Covaci, 2011).

Exposure of humans to PAHs occurs through both air (cigarette smoke, fossil fuel and wood combustion) and food. For non-smokers, exposure to airborne PAHs is highest in densely-populated urban areas and in rural areas where wood and coal are frequently used. Coal and biomass burning for cooking and heating in developing countries has been shown to lead to high indoor concentrations of PAHs. However, food appears to be the major source of PAH intake in industrialized countries, with grilled or charred meats, smoked food, contaminated cereals and vegetables as major sources (Srogi, 2007).

For metals, food and drinking water are the major exposure routes in the general adult population. For small children, however, normal hand-to-mouth activity can lead to considerable intake of metal-contaminated dust or soil. In addition, children eat, breathe and drink more per body weight than adults, and often have unique dietary patterns (e.g. consume more of a particular food group) that can increase

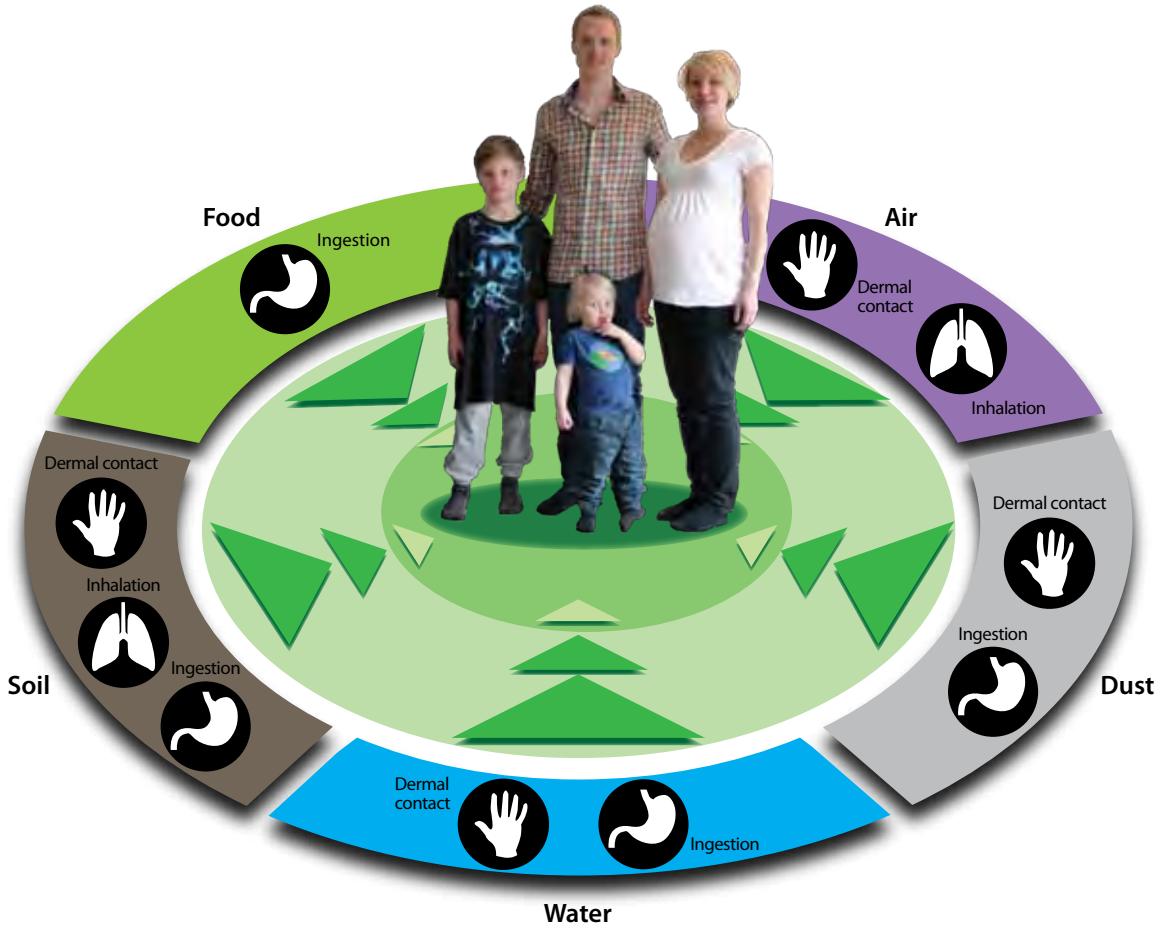


Figure 3.8. Routes of human exposure to EDCs, with each source showing the pathways of EDC uptake.

their exposure to chemicals in those products (WHO, 2007b; National Research Council, 1993). The exposure routes and toxic effects of metals are often dependent on the chemical form (speciation). For example, in contrast to inorganic arsenic species the organic arsenic compounds abundant in seafood are not toxic and are rapidly excreted. Consumption of contaminated ground water is a major source of human exposure to arsenic, with concentrations exceeding the WHO drinking water guideline value of 10 µg/L (IPCS, 2001) in several regions, notably Bangladesh and India (Bhattacharya et al., 2007; Smith, Linga & Rahman, 2000). For mercury, the organic species methylmercury is of major concern as it is accumulated up through food webs, crosses the blood-brain barrier, and is a potent neurotoxic agent (WHO 2010b; 2007c; IPCS; 1990; Chapter 2.6). For this metal, fish consumption is the main exposure route for humans, and many countries have national fish consumption advisories to reduce human exposure.

Exposure to halogenated and non-halogenated phenolic EDCs can occur via intake of food with such chemicals added as antioxidants, e.g. butylated hydroxyanisole (BHA), or through intake of food contaminated with phenolics (bisphenol A, PCP, TBBPA). However, food is possibly not a major pathway of anthropogenic phenolic compounds since their physical-chemical characteristics prohibit accumulation in consumed muscle tissues. Uptake of phenolic EDCs can occur via the skin when they are present in personal care products. Some of the phenolic chemicals are metabolites of POPs that are bound to, and accumulated in, blood and in other tissues (liver, lungs) of animals, i.e. proteinophilic compounds, that are ingested via food. The major proportions of these chemicals in vivo are, however, formed internally.

Food is regarded as the main source for current-use pesticide exposures in the general public. However, pesticides can also be inhaled and absorbed through the skin, particularly by people handling them directly during pesticide application or indirectly when the crop is harvested or processed. The former, in particular, could lead to high exposures.

Human exposure to pharmaceuticals can occur via drinking water, and may be most relevant in areas with extensive pharmaceutical industries and poor drinking water management. The presence of pharmaceuticals in drinking water depends on the source of the water (surface or ground) and its level of treatment (WHO, 2011b).

3.1.5.1 Case study: Exposure from E-waste

Waste electrical and electronic equipment, also commonly referred to as e-waste, describes end-of-life electrically-powered devices, including electronic products such as computers, television sets and cell phones as well as non-

electronics such as refrigerators and ovens. E-waste contains a large number of different chemicals in the plastic and metal components, and several are known or suspected to interfere with the endocrine system of humans and wildlife. More specifically, most e-waste contains a wide range of inorganic contaminants including lead, cadmium, and mercury, and organic pollutants such as PCBs, BFRs and the plastic additives phthalates (Brigden et al., 2005; Puckett et al., 2002; Robinson, 2009). The annual global volume of e-waste is estimated at 20–50 million tonnes and growing at a rate of 3%–5% (UNEP, 2005). The major producers of e-waste are the USA, European Union, China and Japan (Robinson, 2009), but the contribution by developing regions is forecast to increase dramatically in the next decade (Yu et al., 2010).

Despite the existence of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal since 1992 (UNEP, 2009b), it is estimated that 50%–80% of the e-waste collected for recycling has been exported to developing countries (Puckett et al., 2002). China is the largest recipient of exported e-waste (Liu, Tanaka & Matsui, 2006); others include India, Pakistan (Puckett et al., 2002), Vietnam, the Philippines (Terazono et al., 2006), Nigeria and Ghana (Puckett et al., 2005). Uncontrolled e-waste disposal and crude recycling techniques such as open burning of circuit boards and wires, acid-stripping of metals, and plastic chipping and melting (Wong et al., 2007) result in environmental release of not only contaminants contained in e-waste but also of by-products of the recycling and/or incomplete burning processes such as PCDD/Fs (Leung et al., 2007), their brominated and mixed halogenated homologues (Tue et al., 2010), PAHs and halogenated PAHs (Ma et al., 2009), as well as other EDCs (Owens et al., 2007).

Large quantities of e-waste end up in developing countries, where no infrastructure or protocols to safely recycle and dispose of hazardous e-waste exist, nor legislation dealing specifically with e-waste flow (Caravanos et al., 2011; Frazzoli et al., 2010). In Nigeria about 200 tons of e-waste materials were abandoned at riverbanks in dumping sites, where they are manually disassembled, working pieces are repaired and marketed, and useless junk ends up in open fires or in dumpsites (Frazzoli et al., 2010). The e-waste is recycled in a crude way, primarily involving manual disassembly and open burning to isolate copper from plastics. Some of the work is carried out by children, commonly using only rudimentary tools and with no protective equipment. Overall, there is high local contamination of the environment and people in areas with uncontrolled e-waste disposal and recycling.

Soils near e-waste dumpsites in developing countries are known to have high contamination. For example, soils at informal e-waste recycling sites in Asia have been extensively monitored for the occurrence of chemicals including several EDCs. Extremely high levels of lead, chromium, cadmium, PCBs, PBDEs, PCDD/Fs and PAHs were, e.g. reported in soils at an open e-waste burning area in Guiyu (China) (Wong et al., 2007). Elevated levels of antimony and mercury were also found in soil at an Indian e-waste recycling site (Ha et al., 2009). Other studies investigated dust samples and found high abundances of polybrominated and mixed halogenated dibenzofurans (PBDFs and PXDFs) at a Vietnamese e-waste recycling site (Tue et al., 2010b) and elevated chlorinated PAHs in Taizhou (China) (Ma et al., 2009).

Water and foodstuffs in the areas surrounding e-waste recycling sites in developing countries also become contaminated. In villages situated along the rivers where piles of e-waste are disposed of and burned, people use the river water directly for drinking, cooking and washing (Frazzoli et al., 2010). Fish collected near a recycling site in Qingyuan, China accumulated PCBs and PBDEs up to 16,500 and 1100 ng/g wet weight, respectively (Wu et al., 2008), whereas water birds in the same area accumulated these chemicals up to 120,000 and 2200 ng/g fat, respectively (Luo et al., 2009). In Taizhou, rice was found to be contaminated with lead and cadmium, at 2–4 times higher levels than the limit allowed in foodstuffs (0.2 mg/kg) (Fu et al., 2008), and chicken contained elevated PBDEs (up to 18 µg/g fat)(Liang et al., 2008).

High exposure levels of contaminants related to e-waste have been reported for workers and residents in e-waste handling areas and they are exposed from food, water and air. In Ghana, personal air samples collected from e-waste site workers and the environment revealed elevated levels of aluminium, copper, iron, lead and zinc (Caravanos et al., 2011). The blood levels of lead and cadmium in Guiyu children (mean 130 and 16 µg/L, respectively) (Zheng et al., 2008b) are higher than the levels known to cause neurodevelopmental deficiencies (10 and 0.6 µg/dL fat, respectively) (Chen et al., 2011). In the same region, a high exposure level of neonates to chromium (median 94 µg/L in umbilical cord blood) was associated with DNA damage (Li et al., 2008). Regarding BFRs, the PBDE exposure levels in Guiyu are among the highest ever reported (median 600 ng/g fat in human serum) (Bi et al., 2007). High PBDEs levels in women, independent of exposure source, will result in exposure of the fetus via the placenta and of the baby through nursing (Frederiksen et al., 2009; 2010a; Thomsen et al., 2010a; 2010b). The exposure to e-waste generated contaminants, such as dioxins and related compounds, is also significant. Relatively high PCDDs/Fs are found in mothers' milk from Taizhou (21 pg WHO-TEQ/g fat) (Chan et al., 2007). The daily intake of PCDD/Fs by Guiyu residents from inhalation alone was estimated at 1.8−5.8 pg WHO-TEQ/kg/day (Li et al., 2007), comparable to the WHO tolerable daily intake dose. Adverse health effects related to PBDEs, PCBs and other chemicals from e-waste recycling have not been well studied, but DNA damage (Wen et al., 2008) and hypothyroidism (Zhang et al., 2010) have been suggested.

The Partnership for Action on Computing Equipment (PACE) launched in 2008 is a multi-stakeholder group that provides a forum for governments, industry leaders, non-governmental organizations and academia to tackle the environmentally-sound management, refurbishment, recycling and disposal of used and end-of-life computing equipment (PACE, 2012).

3.1.6 Conclusions

EDCs

- The number of identified chemicals with ED properties has increased dramatically between 2000 and 2012.
- EDCs have diverse chemical and physical properties. Many are produced by humans while others are naturally-occurring in the environment. Some are POPs, a dominant class of EDCs known ten years ago, but the more recently-identified ones tend to be less persistent and less bioaccumulative, such as current use pesticides and plasticizers.
- Numerous EDCs have structures that resemble naturally-produced hormones. In addition, some chemicals can affect the endocrine system in their original form while others are more endocrine active after they are transformed in the body or environment.

Sources and environmental fate of EDCs

- EDCs are present in materials (i.e. packaging), goods (i.e. electronics, furniture, household cleaners), personal care products (i.e. cosmetics, lotions, soaps, shampoos), and pharmaceuticals (typically the active pharmaceutical ingredient).
- EDCs are released to water, soils, and the atmosphere during the production, use and disposal of materials and goods, during food production and processing, and through natural processes.
- The sources of EDCs to wildlife and humans are very diverse and include both point (e.g. effluent discharges) and non-point (e.g. agricultural runoff, urban emissions, long-range transport via wind and ocean currents) sources.
- EDCs have a range of fates in the environment. Some are persistent and will concentrate in soils, sediments or fatty tissues while others are more soluble in water and rapidly broken down.

Exposures

- Wildlife are exposed to EDCs in their diet, and through inhalation and dermal absorption.
- Wildlife living downstream of sewage treatment works discharges are exposed to many different EDCs including active ingredients in pharmaceuticals and additives in personal care and cleaning products.
- Wildlife higher on the food chain are particularly at risk for exposure to POPs and other similar chemicals with ED properties due to their biomagnification through the food web.
- Humans are exposed to EDCs from multiple sources including ingestion of food, dust and water, inhalation of volatile and particle bound contaminants, and dermal uptake.
- Disposal and recycling of e-wastes in developing countries has been identified as a source of EDC exposure for humans and wildlife.
- EDCs found in food include POPs, pesticides, additives in food packaging, metals, and PAHs.
- EDCs are present in personal care products, and their uptake through skin has been recently recognized as a significant route of human exposure.
- Some EDCs, like flame retardants used in furniture and other consumer products, are found at high concentrations in household dust. Young children can have higher exposures to EDCs than adults because of their hand-to-mouth activities and because they play close to the ground.
- Wildlife and humans are exposed to very complex mixtures of EDCs.

3.2 The EDCs found in wildlife and humans

3.2.1 Wildlife

3.2.1.1 Internal exposure

Distribution in the body **-** Once an EDC is taken up into the body of an invertebrate, fish, bird or mammal from the water or its diet, the EDC can be transported to different tissues, where it can be metabolized, excreted, or stored. Storage of EDCs occurs in the fatty tissues (liver, brain, adipose), in proteins (liver, muscle), and in the bones. Most POPs are lipophilic and stored in fatty tissues of wildlife. However, when fats are mobilized and used by a fish or bird to produce eggs, by mammals to produce milk to nurse offspring, or as a source of energy during periods of low food or starvation, this leads to higher concentrations of POPs in circulating media (blood) which may have adverse effects on the organism. Other EDCs are proteinophilic (e.g. perfluorinated chemicals, methylmercury) and are found at the highest concentrations in protein-rich tissues like liver.

There is also placental transfer of EDCs (e.g. POPs) to the fetus of wild mammals and this can cause effects in the young. Maternal transfer to the fetus is known to occur for PBDEs, mercury, organochlorine pesticides, and plasticizers and can result in a number of effects on the reproductive system of the offspring (see review by Hamlin & Guillette, 2011; see below under 3.2.2 and Chapter 2.2, 2.3 & 2.6).

Metabolism and excretion **-** There are several enzymes in wildlife that transform EDCs to metabolites. For vertebrates the liver is the main site of metabolism of many of the EDCs and this process typically makes it easier for an organism to remove the EDC from its body in faeces and urine. However, metabolism can also make an EDC more harmful (e.g. hydroxylated PCBs interfering with the transport of thyroid hormones in blood; Bergman, Klasson-Wehler & Kuroki, 1994; Letcher et al., 2000; see Chapter 2.5).

EDCs that have an affinity for fats are deposited into the high-fat eggs of birds and fish, where they can have an effect on the hatching and survival of the offspring (Hamlin & Guillette, 2011). Similarly, milk is high in fats and is a source of EDC exposure for nursing young due to the transfer of lipophilic EDCs like PCBs from the mother into her milk. For example, for High Arctic, Canadian subpopulations, polar bear cubs have higher levels of POPs than their mothers (Polischuk, Nordstrom & Ramsay, 2002), likely due to high exposure of the cubs during nursing.

3.2.1.2 What has been measured in wildlife

Over the last decade the understanding of the types and levels of EDCs found in wildlife has improved. While studies continue to focus on POPs, for example, in Arctic wildlife (e.g. Letcher et al., 2010), a broader range of chemicals are now known to occur in wildlife in remote and urban environments. In these environments, EDCs in some species, especially the long-lived top predators, can exceed levels known to cause effects.

Blood, milk, bile, fat, brain, and muscle tissues (or whole bodies of small organisms like mussels) are used to measure the internal dose of an organism to EDCs. These analyses provide information on what is getting into the body and can be more easily linked to effects on the endocrine system. However, some EDCs are very short-lived in the body and difficult to measure, or they will cause effects that appear later in life, making it impossible to link exposure to adverse responses.

The most well studied EDCs in wildlife tissues are the POPs, including DDT (and its main transformation product DDE), chlordanes, dieldrin, PCBs, "dioxins" (i.e. PCDDs/PCDFs and some dioxin-like PCBs), PBDEs, PFOS and PFCAs. In the previous document much of the discussion focused on PCBs and DDTs, PBDEs were only briefly mentioned, while PFOS and PFCAs were not mentioned at all (IPCS, 2002). The main POPs selected for discussion in this chapter are PCBs, PFOS, and PBDEs (select data are shown in sections 3.2.1.3 and 3.2.1.4). Other POPs with large tissue residue datasets include HCB, chlordane-related compounds, dieldrin/endrin, toxaphene-related compounds, mirex, and HCH isomers (in particular β-HCH). Some data exist for other POPs such as pentachlorobenzene, endosulfan, hexabromobiphenyl, and chlordecone in invertebrates, fish and other wildlife but they are not as comprehensive. Similarly, more limited datasets exist for other persistent organohalogen compounds, which are not on the Stockholm Convention POPs list such as chlorinated paraffins, polychlorinated naphthalenes, octachlorostyrene, pentachlorophenol/anisole, and methoxychlor.

The levels of POPs in wildlife vary from one location to another and are highly dependent on the extent of local contamination. For example, DDT (as 4,4'-DDT and 4,4'-DDE) levels were measured in fish and domestic animals in an area of South Africa where DDT spraying is on-going for malaria mosquito vector control. Average concentrations of DDT (on a fat weight basis) in tilapia were 4 to 6 times higher downstream of a sprayed area than at an upstream site (Barnhoom et al., 2010). Similarly, levels of DDTs in the fat of chickens were much higher (up to 700 times) from the sprayed than non-sprayed villages (Van Dyk et al., 2010).

Some of the chemicals used in pharmaceuticals and personal care products are being found in the tissues of wildlife living near sewage treatment works outfalls. The chemicals from personal care products include benzotriazole UV stabilizers, parabens, triclosan, and organophosphorous compounds (Dann & Hontela, 2011; Kim et al., 2011). Triclosan has been found in a range of aquatic organisms including algae, invertebrates, fish and dolphins (Dann & Hontela, 2011). A number of pharmaceuticals have also been found in wildlife in highly populated areas including the antiepileptic carbamazepine and the active ingredients of several antidepressants (fluoxetine, sertraline, venlafaxine, citalopram, norfluoxetine, diphenhydramine, diltiazem) in muscle or liver of wild fish or fish caged downstream of wastewater outfalls (Brooks et al., 2005; Metcalfe et al., 2010; Ramirez et al., 2009; Schultz et al., 2010, Bringolf et al., 2010). In addition, human contraceptives have been found in fish muscle at low parts per billion levels ((EE2; Al-Ansari et al., 2010) and in the plasma of fish exposed to municipal wastewaters (levonorgestrel up to 12 ng/mL; Fick et al., 2010). Less is known about whether pharmaceuticals and personal care products accumulate in terrestrial organisms. Some studies have shown that earthworms take up triclosan from soils that were treated with solids from municipal wastewater treatment plants or biosolids (Kinney et al., 2008).

There have been measurements of mercury in wildlife for over 40 years and, thus, there is extensive literature on this EDC (Das et al., 2003; Law 1996; Reijnders, Aguilor & Borrell, 2009; Thompson, 1996). Large databases for mercury in fish muscle are available (UNEP, 2002; US EPA, 1999a). Fish accumulate mercury primarily as methylmercury rather than other chemical forms of mercury (National Research Council, 2000; Scheuhamer et al., 2007). Fish-eating marine mammals and birds are therefore mainly exposed to methylmercury, because almost all of the mercury present in their diet is methylated. In tissues such as skeletal muscle, fur, feathers, and eggs, methylmercury is usually the predominant form (Endo et al., 2005; Scheuhammer, Wong & Bond, 1998).

PAHs have been widely measured in wildlife, often as part of monitoring responses to oil pollution events (Hellou, 1996). In marine mammals, levels of these compounds in blubber are generally lower than POPs (Hellou, 1996, Reijnders, Aguilor & Borrell, 2009). Lower molecular weight (2–4 ring) PAHs predominate in most marine mammal blubber samples (Fair et al., 2010; Taniguchi et al., 2009). Relatively high ΣPAH concentrations (on a fat weight basis) have been reported in blood. For example, sea otters from Alaska and the California coast had average Σ_{26}PAH in blood serum ranging from 3.1 to 9.8 μg/g fat (Jessup et al., 2010). Sea otter livers from the same regions had similar Σ_{16}PAH (16 unsubstituted "priority" PAH) concentrations when expressed

on a fat weight basis (Kannan et al., 2008). Highest Σ_{16}PAH were from sea otters collected in Prince William Sound, the site of the Exxon Valdez oil spill. There has been limited study of PAHs in seabird tissues. Measurements of Σ_{16}PAH in seabird livers from the Mediterranean and Eastern Atlantic found at low ng/g wet weight concentrations (Roscales et al., 2011). Seabird eggs have also been shown to generally have low PAH concentrations, with few distinctive differences between geographic areas (Pereira et al., 2009; Shore et al., 1999). Seabird fat from King George Island (Antarctica) had ΣPAHs (20 unsubstituted 2-6 rings + methyl naphthalenes) ranging from 1.5-5.7 µg/g fat, with naphthalene and methyl naphthalenes predominating. Phenanthrene was the most abundant unsubstituted PAH in seabird eggs from the UK coast, while methylnaphthalenes predominated in most other locations (Pereira et al., 2009). Seabird blood has been used as a bioindicator of PAH exposure from oil spills (Pérez et al., 2008). Higher plasma concentrations of Σ_{16}PAH were found in oil-exposed seabird colonies (Pérez et al., 2008; Troisi et al., 2007).

It is clear that wildlife contain a diverse mixture of EDCs. For example, the broad array of known or potential EDCs that have been detected in fish is illustrated in **Figure 3.9** from the Baltic Sea monitoring programme (HELCOM, 2010). Organotins, bis(2-ethylhexyl)adipate, and phenolics including triclosan and chloro- and bromophenols are the predominant compounds. HCB, PFOS and pentaBDEs are the only POPs measured among the 23 chemicals in these fish.

3.2.1.3 Spatial trends for wildlife

The previous global assessment on the State-of-the-Science of EDCs reviewed the spatial trends of PCBs and DDT-related compounds in wildlife with a focus on the Baltic, the Great Lakes, and the Arctic (see Chapter 6 Annex in IPCS, 2002). Here selected studies published since 2002 that have examined spatial trends of major EDCs in invertebrates, fish and other wildlife at global or large regional scales are discussed. Tanabe & Subramanian (2006) reviewed the regional and global spatial trends of POPs in invertebrates, fish and wildlife, with an emphasis on species that could be used for global biomonitoring. They discussed the essential characteristics of animal bioindicators and the important criteria were: (a) the broad geographical range of the species; (b) known feeding habits; and, (c) ease of sampling and sample processing. Thus, marine mammals such as dolphins (blubber), seabirds (especially their eggs), globally-distributed fishes such as tuna, and mussels have frequently been used to study large scale spatial trends of EDCs. Select studies that have examined broad regional or global trends in concentrations of EDCs in wildlife are discussed here. The vast majority of the studies on fish, mammals and birds are on POPs and not on the wider range of known or potential EDCs (see **Table 3.1**). This reflects the fact that vertebrate wildlife are generally not suitable for biomonitoring of more rapidly metabolized chemicals. Therefore results for selected contaminants in mussels were also included.

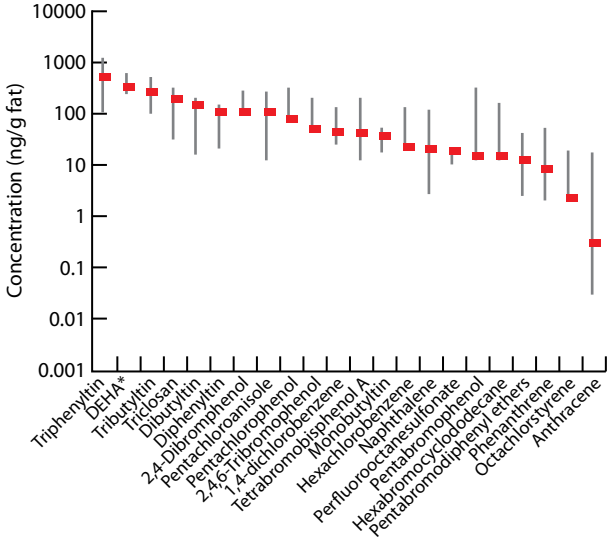


Figure 3.9. Concentrations (ng/g fat) of commonly observed chemicals in muscle of fishes from background areas in the Baltic Sea (HELCOM 2010, original data from Bignert et al., 2006). *DEHA is bis(2-ethylhexyl) adipate.

EDCs in mussels

Over the past 20 years, mussels and other bivalves (e.g. oysters) have been monitored in "mussel watch" programmes in many regions including; the USA, Caribbean, Central, and South America (Barra et al., 2006; Farrington & Tripp, 1995; Kimbrough et al., 2008); Japan, Korea, China, Vietnam and India (Ramu et al., 2007; Tanabe & Minh, 2010); Australia (Prest et al., 1995); the Persian Gulf (ROPME, 2011); the Baltic (HELCOM, 2010); the North Sea and northeast Atlantic (OSPAR, 2009); and the Mediterranean including north Africa (Scarpato et al., 2010). Only sub-Saharan Africa, and parts of the Russian Arctic, Alaskan and Canadian Arctic have not been included in various studies and this may reflect an absence of suitable species. While most of the monitoring has been on metals and POPs, PAHs have been included in many of these programmes (Farrington & Tripp, 1995; HELCOM, 2010; OSPAR, 2009) so that spatial trends in exposure to PAHs of coastal marine environments can be assessed over a broad area. Similarly, spatial trends of TBT have been assessed in the Baltic (HELCOM, 2010), the Northeastern Atlantic (OSPAR, 2009), and in East Asia (Sudaryanto et al., 2002; Choi et al., 2009). Recently the use of mussels has been extended to alkyl phenols, phthalates, bisphenol A, and triclosan (Gatidou, Vassalou & Thomaidis, 2010; Sánchez-Avila et al., 2011). Collectively these studies have shown that EDC concentrations in mussels vary considerably from one location to another and are typically highest in areas where their use and release is greatest. A large study of mussels from East Asian sites surveyed in Malaysia, Singapore, the Philippines, and Indonesia found concentrations of nonylphenol, octylphenol and bisphenol A that were

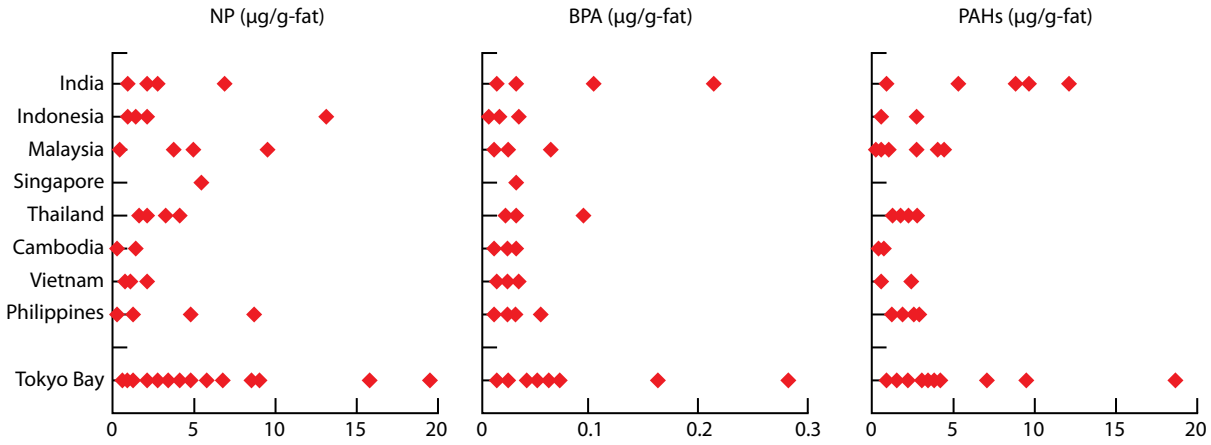


Figure 3.10. Concentrations (µ/g fat) of nonylphenol (NP), bisphenol A (BPA) and PAHs for mussels from South and Southeast Asia and in Tokyo Bay. (Figure based on Isobe et al. (2007)).

comparable to those observed in Tokyo Bay, while much lower concentrations were found in coastal marine areas of Vietnam and Cambodia (Isobe et al., 2007) (**Figure 3.10**).

PCBs and DDTs

The global distribution of PCBs and chlorinated pesticides in skipjack tuna *(Katsuwonus pelamis*) has been reported by Ueno et al. (2003). The geographic coverage of these studies included the Pacific Ocean, the Indian Ocean and the southwestern Atlantic (**Figure 3.11**). Unfortunately the North Atlantic was not included. Nevertheless these studies provide insights into the spatial trends of contamination by POPs in an important food source for humans and in regions with rapidly growing chemical manufacturing and product recycling industries. Elevated concentrations of ΣPCBs and ΣDDTs were found in tuna samples from the western Pacific, more specifically in the south China Sea and Sea of Japan. Relatively high levels of ΣPCBs were also found in tuna collected off the coast of Brazil. In contrast, ΣPCBs were relatively low in samples from the Indian Ocean while ΣDDTs was elevated in the Bay of Bengal region, likely contaminated from use of DDT for agriculture and for malarial vector control.

Many species of marine mammals are highly contaminated by POPs due to their top trophic positions and, in some animals, these chemicals have been linked with population declines (Chapter 2.12). The family *Delphinidae* (dolphins) is globally distributed and there are measurements, mainly in dead/stranded animals, from around the world (Houde et al., 2005). In general, the highest levels of ΣPCBs and ΣDDTs (sum of DDT-related compounds) were found in species inhabiting the midlatitudes of industrialized Asia, North America and Southern Europe (**Figure 3.12**), reflecting the areas where these chemical compounds have been intensively used. Very high concentrations in dolphin blubber are observed, sometimes exceeding 1000 µg/g fat. Possible declining POPs concentrations in dolphins are discussed in section 3.2.1.4.

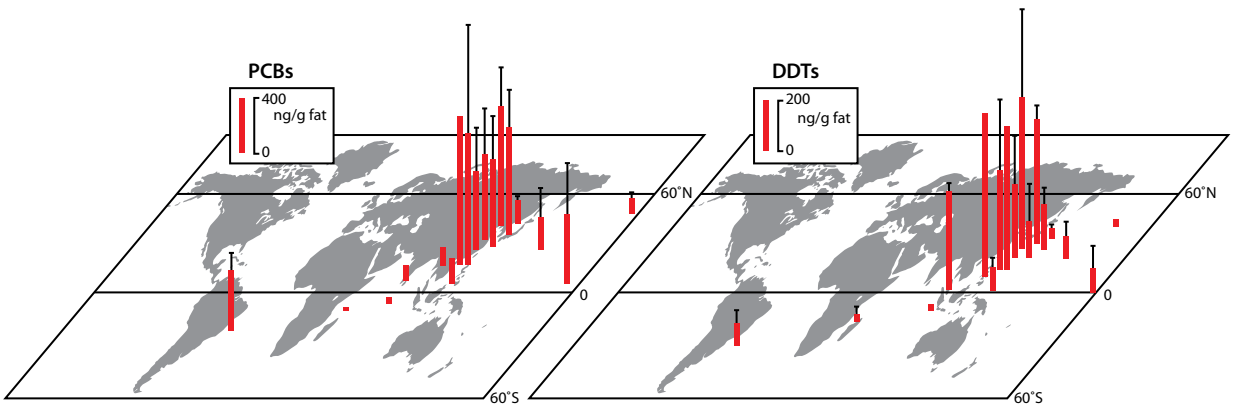


Figure 3.11. Geographical distribution of the sum of 117 PCB congeners and the sum of 4,4'-DDT, -DDE and –DDD concentrations (ng/g fat) in liver of skipjack tuna from east Asian waters, the central North Pacific, Indian and South Atlantic oceans. (based on Ueno et al., 2003).

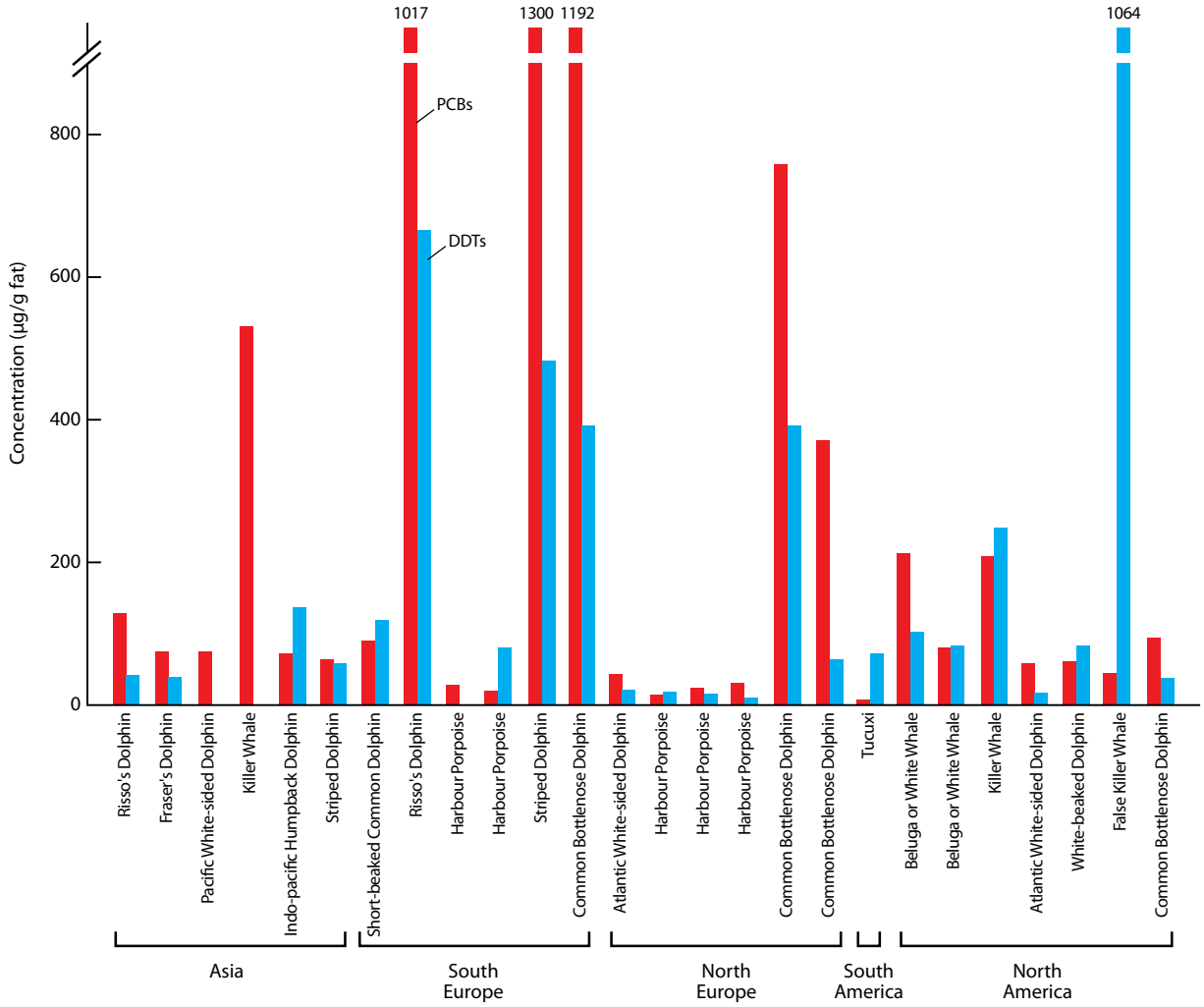


Figure 3.12. Mean concentrations (µg/g fat) of PCBs and DDTs in blubber of stranded male delphinoids from various regions. (based on figure from Houde et al., 2005).

PBDEs

Global contamination by PBDEs has been well documented over the past 10 years and reviews of spatial and temporal trends in wildlife are available (Chen & Hale, 2010; de Wit, Alaec & Muir, 2006, de Wit, Herzke & Vorkamp, 2010; Hites, 2004; Law et al., 2006; 2008b). The major congeners detected are BDE-47, -99, -100, -153 and -154, resulting from the use of PentaBDE and OctaBDE products. These congeners all exhibit significant trophic biomagnification, especially in aquatic food webs with fish and piscivorous birds as top predators.

Skipjack tuna were also used to assess the global distribution of PBDEs and HBCDD (Ueno et al., 2004; 2006) (**Figure 3.13**). Relatively high concentrations of ΣPBDEs and HBCDD were found in samples from the South China Sea and Sea of Japan, areas which are near large urban and industrial areas with known use, recycling and manufacturing sites for BFRs. Relatively high levels of ΣPBDEs were also found in tuna collected off of the coast of Brazil. In contrast ΣPBDEs and ΣHBCDDs were relatively low in samples from the Indian Ocean.

Chen & Hale (2010) examined the global spatial trends of PBDEs in birds (tissues or eggs) and found that North American birds exhibited much higher concentrations of ΣPBDEs than those from Europe and Asia (**Figure 3.14**). Differences in species and also in the number of congeners used to calculate ΣPBDEs did not account for the large regional differences. This geographical difference is consistent with the fact that the North American market has encompassed the bulk of the world's PentaBDE production (BSEF, 2003). Major differences were also apparent between remote and urban regions; the Canadian and European Arctic (Svalbard) and Antarctica were much lower in concentrations when compared to samples collected near urban areas. Chen & Hale (2010) also reviewed the available data on BDE-209 in birds around the world. Highest concentrations were observed in Chinese kestrels and USA peregrine falcons (**Figure 3.15**). The distinctive regional patterns observed with ΣPBDEs are not as apparent for BDE-209, with both North American and East Asian birds having high exposures. This may be

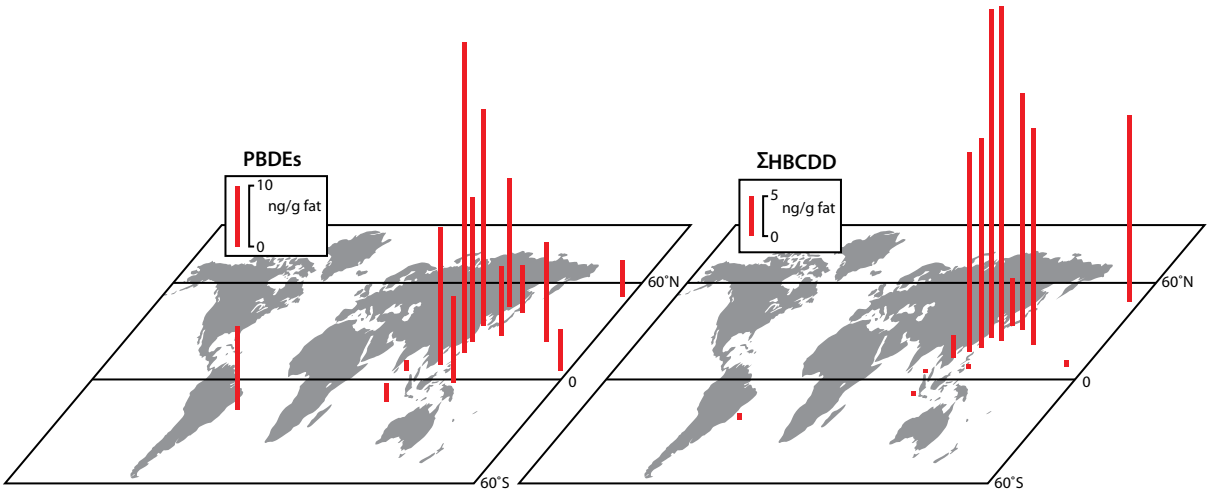


Figure 3.13. Sum of 11 PBDE congeners and sum of α–, β–, γ–HBCDD concentrations (ng/g fat) in muscle of skipjack tuna from east Asian waters, the central North Pacific, Indian and South Atlantic oceans (Ueno et al., 2004; 2006).

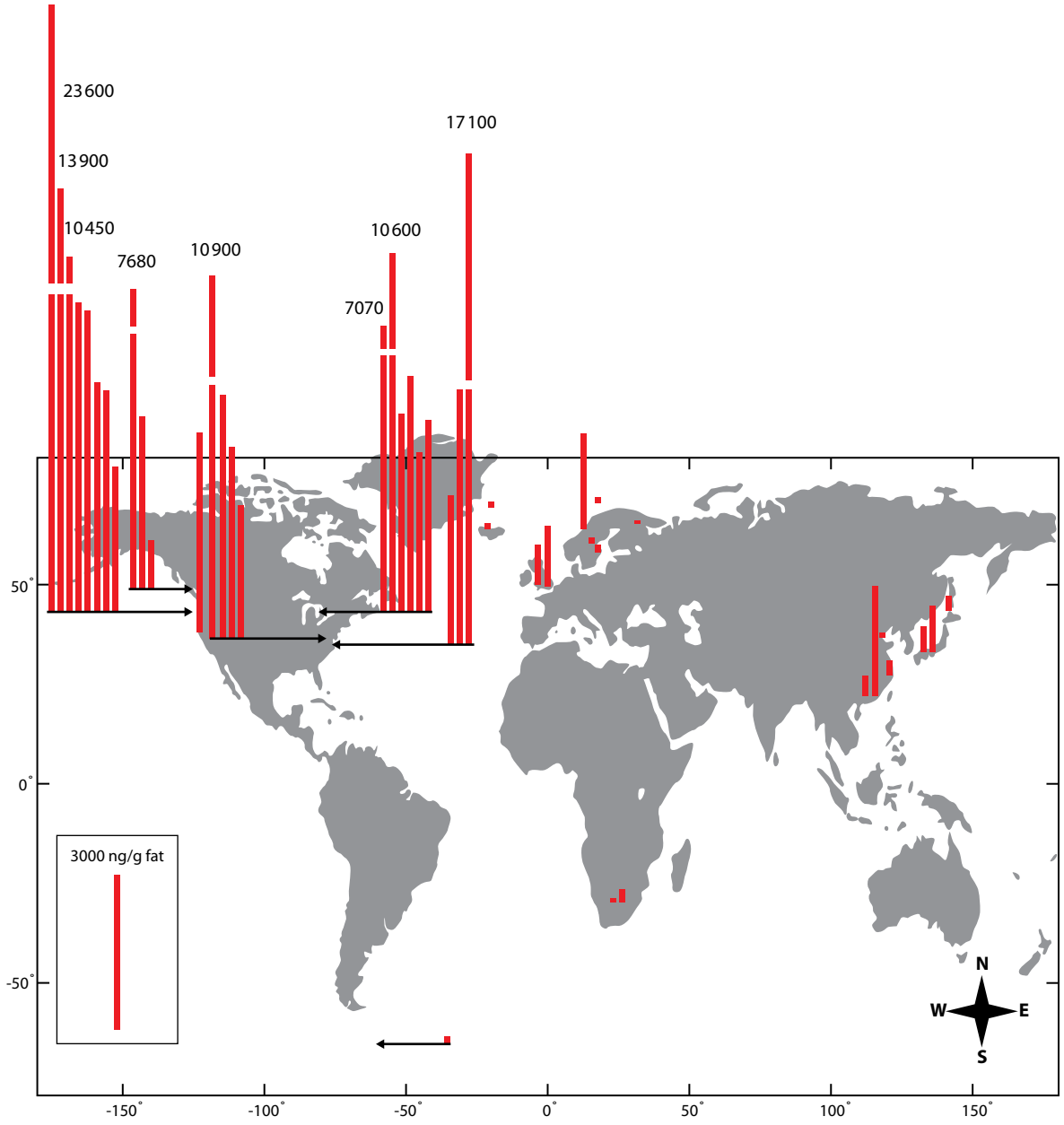


Figure 3.14. PBDE concentrations (ng/g fat) in bird tissues or eggs from North America, northern Europe, East Asia and South Africa (Chen & Hale 2010). (Figure redrawn; Used with publisher's permission)

explained by DecaBDE being the major PBDE product used in Asia, at least in the early 2000s (BSEF, 2003).

PFCs

Knowledge of PFCs in wildlife has also increased over the past decade (reviews by Beach et al., 2006; Houde et al., 2006, 2011); they were not included in the previous global assessment of EDCs (IPCS, 2002). PFOS in wildlife was first reported by Giesy & Kannan (2001). PFOS and related PFCs had been missed in previous surveys of halogenated contaminants in wildlife due to a lack of suitable analytical methodology; the sulfonates could not be readily analysed by gas chromatography, a method used for POPs like PCBs. Also the prevailing view that a halogenated compound with an ionisable carboxylate or sulfonate group would not biomagnify may have led regulators to give these PFCs low priority for exposure assessments. Subsequent reports identified many related compounds such as perfluorohexanesulfonate (PFHxS) and perfluorodecanesulfonate (PFDS), as well as the PFCAs with 8 to 14 carbon chains. Recently a new class of perfluoroalkyl sulfonates, the perfluoroethylcyclohexane sulfonates, was identified in Great Lakes fish and other aquatic biota (de Silva et al., 2011). Houde et al. (2006; 2011) have comprehensively reviewed the reports of PFCs in biota.

An initial large-scale study by Giesy & Kannan (2001) demonstrated the global distribution of PFOS in wildlife. Concentrations of PFOS in animals from relatively more populated and industrialized regions, such as the North American Great Lakes, Baltic Sea, and Mediterranean Sea, were greater than those in animals from remote marine

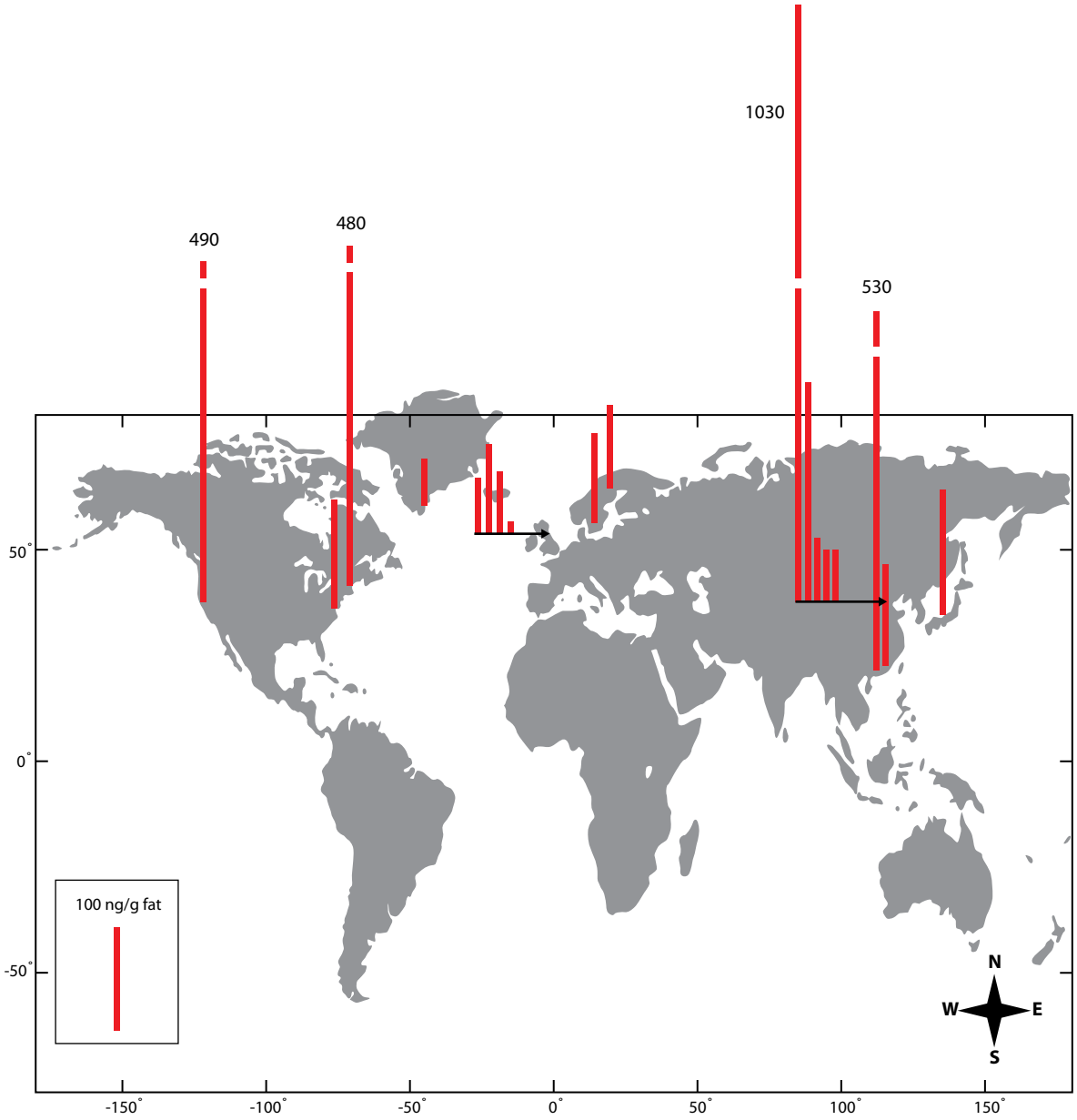


Figure 3.15. BDE-209 concentrations (ng/g fat) in bird tissues or eggs from North America, northern Europe, East Asia and South Africa (Chen & Hale 2010). (Figure redrawn; Used with publisher's permission)

locations, with the exception of polar bears from Alaska which had relatively high concentrations. PFOS remains the predominant PFC found in all species, tissues, and locations analyzed around the world (Houde et al., 2006; 2011). The geographical distribution of PFOS in marine mammals based on studies published in the period 2006 to 2010 is shown in **Figure 3.16**. Consistent with previous results, highest PFOS concentrations were reported for marine mammals in the Baltic and North Seas and in coastal Japanese waters, indicating the importance of urban and industrial sources.

Mercury/methylmercury

Although there is great interest in mercury in the global environment under initiatives such as UNEP's Global Mercury Partnership (UNEP, 2011a), the focus for large spatial comparisons has mainly been on atmospheric emissions, sources and air concentrations (AMAP/ UNEP, 2008). Earlier reviews have summarized the extensive measurements of mercury in marine mammals (Law, 1996), seabirds and terrestrial animals (Thompson, 1996). Das et al. (2003) have summarized mercury and methylmercury concentrations in livers of marine mammals published to about 2001. They noted that levels of mercury in liver varied several orders of magnitude among species and locations. Particularly important are the ages of marine mammals since mercury is usually correlated with age; other factors include diet, trophic level (determined with nitrogen isotope ratios), sex, location and metabolic rate. This applies equally to bird tissues (except eggs) and fish. Thus broad geographic comparisons of mercury concentrations are of little value without the supporting age, size, sex and diet information.

Total mercury in marine mammal and seabird tissues from the Arctic have been reviewed as part of the Arctic

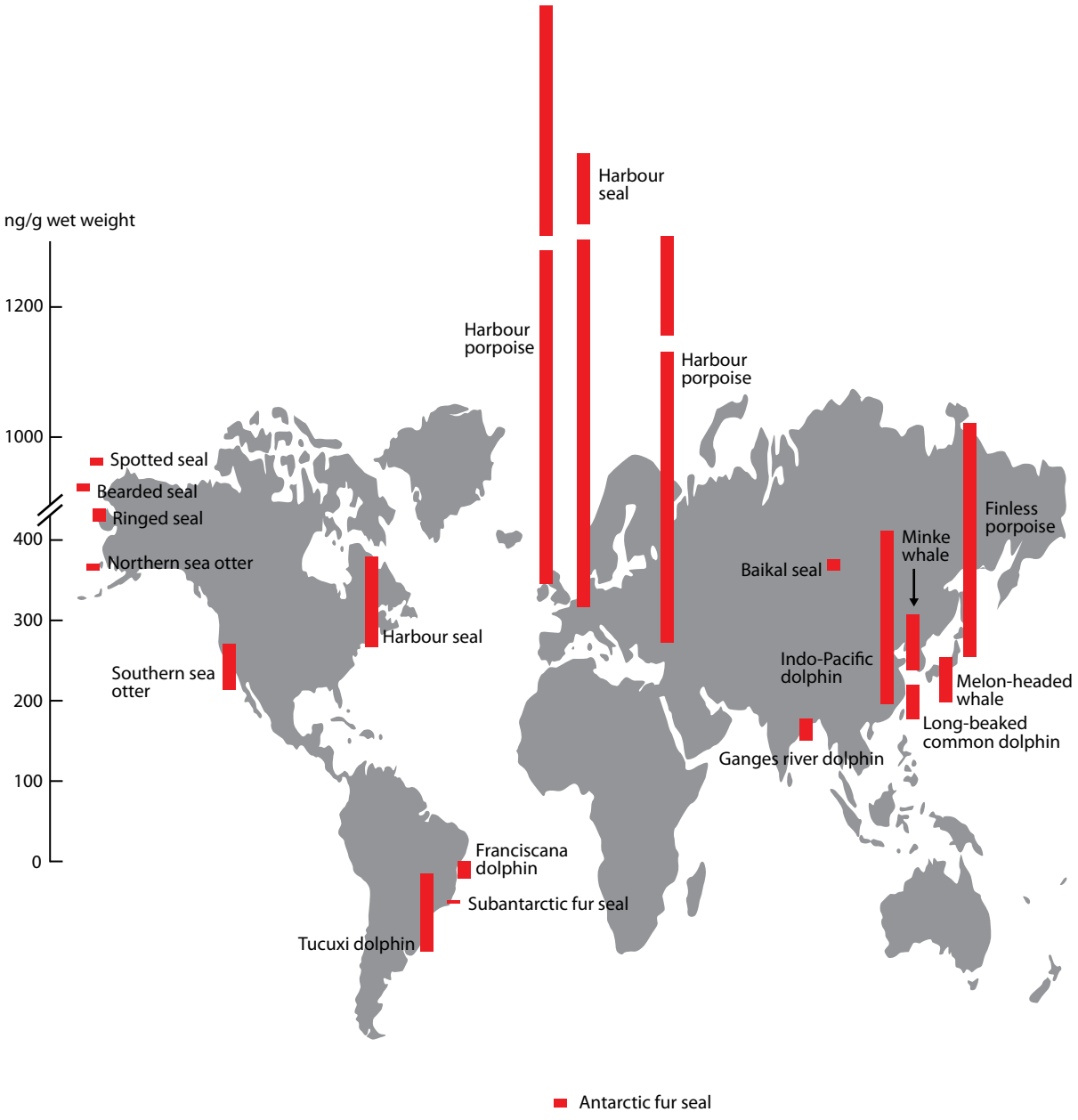


Figure 3.16. PFOS concentrations (ng/g wet weight) in liver of marine mammals worldwide (based on figure from Houde et al., 2011).

Monitoring and Assessment Programme (AMAP) (Dietz, Pacyna & Thomas, 1998; Ford et al., 2005; Riget et al. 2011). The use of common sampling and analysis protocols within the program allowed for geographic comparisons after adjustment for factors such as age. For example, spatial trends of mercury in ringed seal liver were compared using means adjusted to five year old animals (**Figure 3.17A**). Riget et al. (2005) used the same dataset, as well as samples from the White Sea in northwestern Russia, to study longitudinal trends of mercury in ringed seals (**Figure 3.17B**). Very high concentrations of total mercury were present in seals in the western Canadian Arctic but not further west at Barrow, Alaska, USA Natural geological differences in mercury may be important, although differences in diets and trophic levels of ringed seals in Hudson Bay and the Beaufort Sea could also explain some of the differences. Recent comparisons of mercury in polar bears from these two regions suggest that longer food chains (i.e. more trophic levels) and higher water concentrations of methylmercury best explain site differences (St Louis et al., 2011).

3.2.1.4 Temporal trends for wildlife

While information on spatial trends of EDCs in biota is available from a large number of global and regional monitoring programmes and literature reviews, there is much less information on temporal trends. The long term temporal trend programmes in the Baltic, the Great Lakes and the Arctic, which were highlighted in the 2002 IPCS assessment of EDCs (IPCS, 2002), continue to be the longest datasets for many POPs and mercury. Here some previous time trends cited in the 2002 EDC assessment are revisited and, where possible, expanded to include other regions and more chemicals.

PCBs and OC pesticides

Long term trends of POPs (PCBs and DDTs) in guillemot eggs have been studied at St. Karlsö (Gotland Island, Baltic Sea) since 1969, and archived samples from the late 1970s to present have been analyzed for many other halogenated organics and metals (Bignert et al., 2010). The common guillemot (*Uria aalge*) is a fish-eating bird that nests in a few remote colonies. These colonies stay in the Baltic region all year and thus guillemot eggs are representative of marine contamination in the Baltic. Time trends for PCBs (sum of 9 congeners), 4,4'-DDE, β-HCH and HCB in guillemot eggs are shown in **Figure 3.18**. All 4 legacy POPs continue to decline at about 5-10% per year. These declines parallel global declines in estimated emissions of these POPs (see inset graphics) and recovery of bird and mammal populations that were affected by their exposure (Chapter 2.12).

Many temporal trend datasets exist for POPs in Arctic wildlife (Riget et al., 2010). POPs in polar bears from East

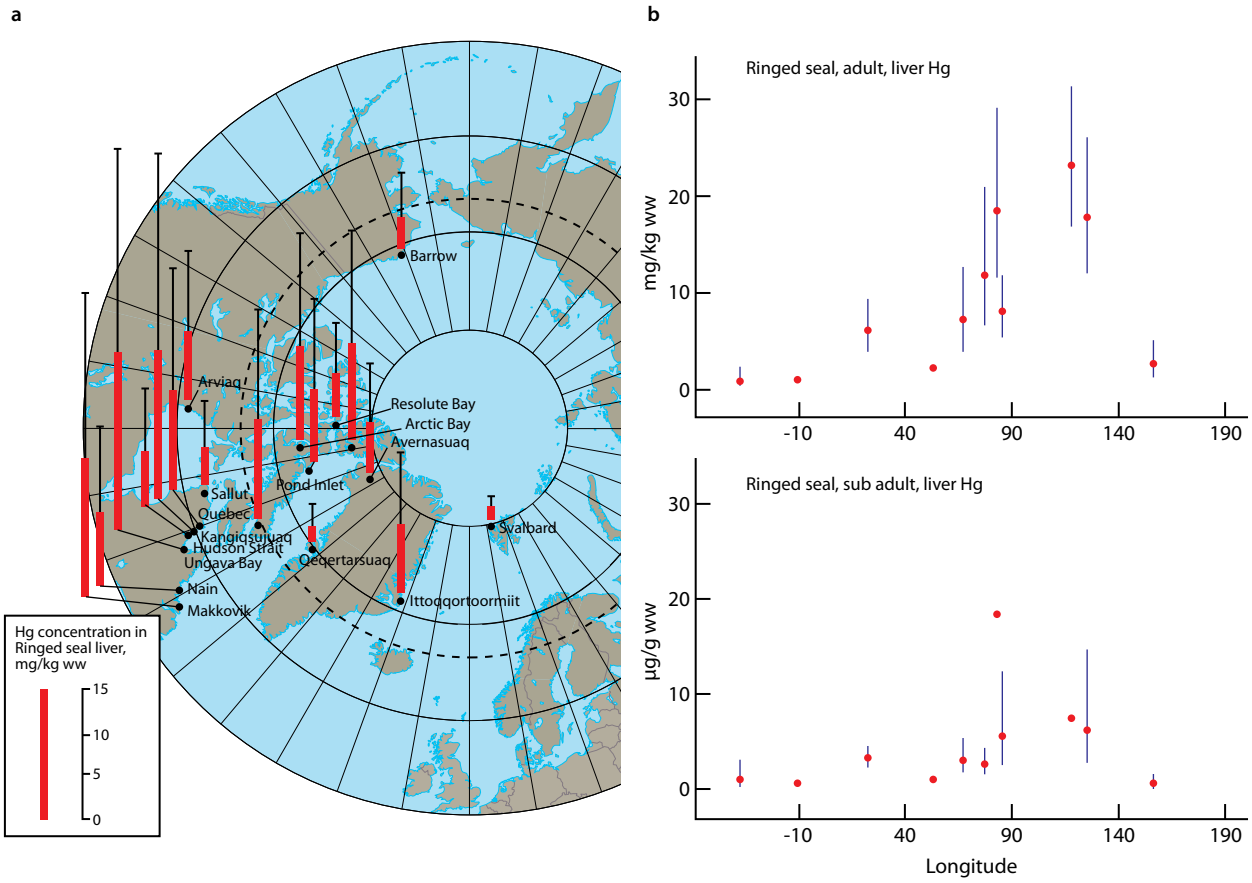


Figure 3.17. a. Mean concentrations (mg/kg wet weight) of total mercury in adult ringed seal liver from Greenland, northern Canada and Alaska based on sampling over the period 1995-2000. Data are mean-adjusted to five-year old animals (Ford et al., 2005). b. Trends of liver mercury in age-adjusted adult (top panel) and subadult seals (bottom panel) with longitude (Riget et al., 2005). Composite figure based on the references given.

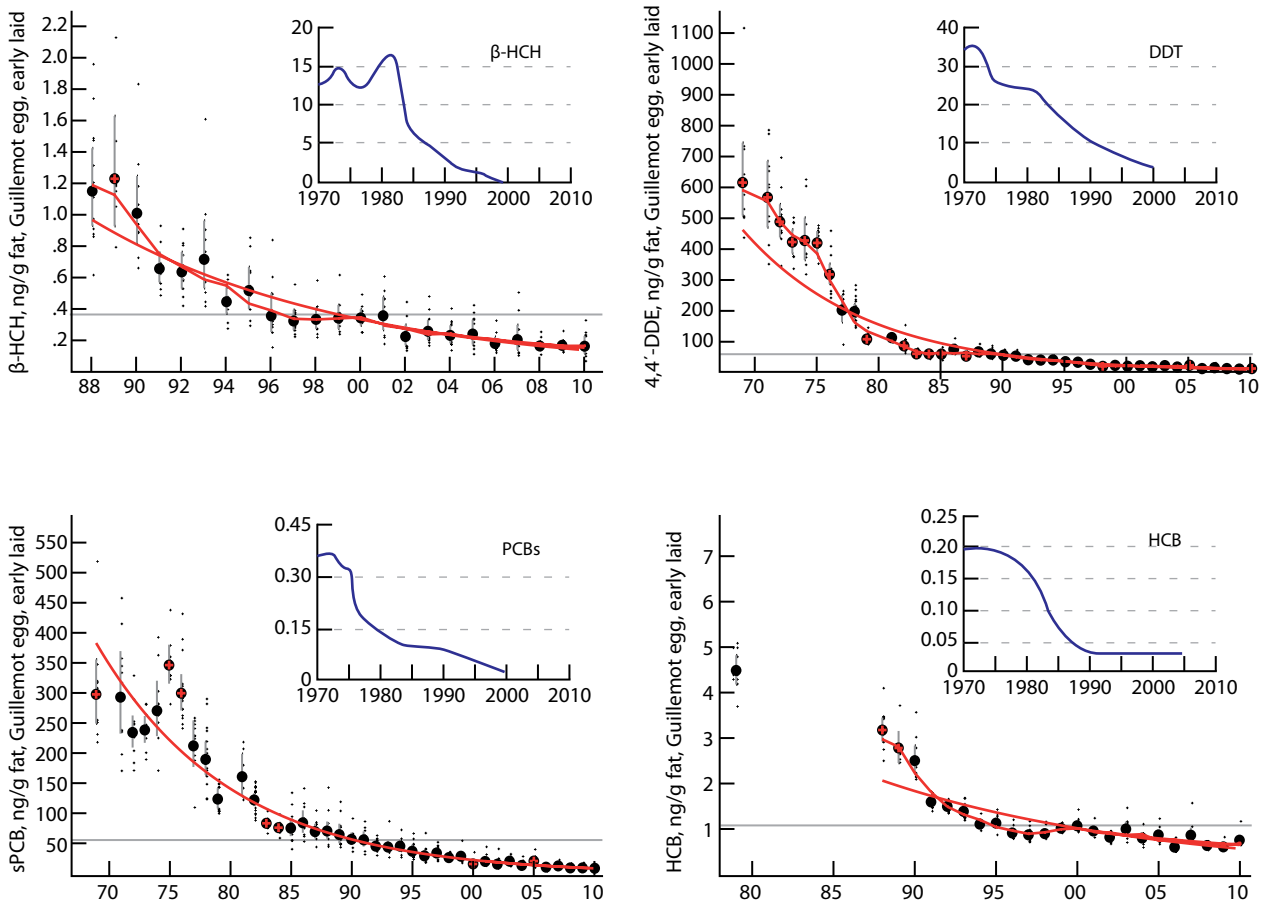


Figure 3.18. Temporal trends in concentrations (ng/g fat) of β-HCH, 4,4'-DDE, ΣPCB, and HCB in guillemot eggs (first laid) from Gotland (Baltic Sea), a long term monitoring site for the Swedish National Marine Monitoring Programme (Bignert et al., 2010). Estimated global emissions (kT) of PCBs (Breivik et al., 2002), 4,4'-DDT and β-HCH (Li & Macdonald, 2005) and European emissions of HCB (Pacyna et al., 2003) are shown in smaller inset graphics.

Greenland are among the longest time series in the Arctic. As top predators in the marine food web, polar bears are excellent biomonitoring species for chemicals that biomagnify. The East Greenland population has a well-defined and large home range away from local sources (Dietz et al., 2012). Samples are available from an annual hunt by the Inuit. PCBs have declined at an average rate of 3.8%/year in juvenile bears over the period 1983 to 2010 (**Figure 3.19**). A decline averaging -4.3 % for 4,4'-DDE, -3.5% for β–HCH and -2.0% for HCB per year was observed in juvenile bears. In contrast, no consistent decline was observed in adult female or male bears, reflecting the long half-lives of chlorinated POPs in these long-lived mammals.

Tanabe & Minh (2010) have reviewed the long term trends of PCBs and chlorinated pesticides in marine biota in the Asia-Pacific region. They noted that concentrations of POPs in marine mammals from inland and coastal areas in Japan and China, including Hong Kong, have decreased in comparison with their severely polluted status in the 1970s. On the other hand, species such as minke whale in Antarctica showed little change over time, reflecting the long residence time of these contaminants in open ocean food webs. Time trends of POPs in blubber of stranded striped dolphins from Gogo-shima and Taiji in southwestern Japan from a study by Isobe et al. (2009) are shown in **Figure 3.20**. The striped dolphin is an ocean-going species and may reflect contamination from coastal China as well as Japan. Thus, it is interesting that the temporal trends of POPs are quite different from those of top predators in the Baltic or the Arctic, with relatively steady levels of PCBs and no significant decreasing trends in ΣDDTs and ΣHCH concentrations, suggesting continuing discharge of these contaminants. Chlordane, which had been used as a termiticide for mostly wooden houses in Japan and possibly other locations along the east Asia coast, showed an increasing trend (statistically significant) even after the ban on these chemicals in 1986 (Isobe et al., 2009), possibly due to its ongoing release from older sites of use.

From reviews of information for Asia, it is also apparent that the ability to detect change (statistical power) in current temporal trend datasets is low, due to the small number of sampling years and a lack of annual sampling (Bignert et al., 2004). However it is bound to improve with additional sampling years given the availability of specimen banks (Tanabe, 2006). Although there are substantial numbers of studies on POPs in South America

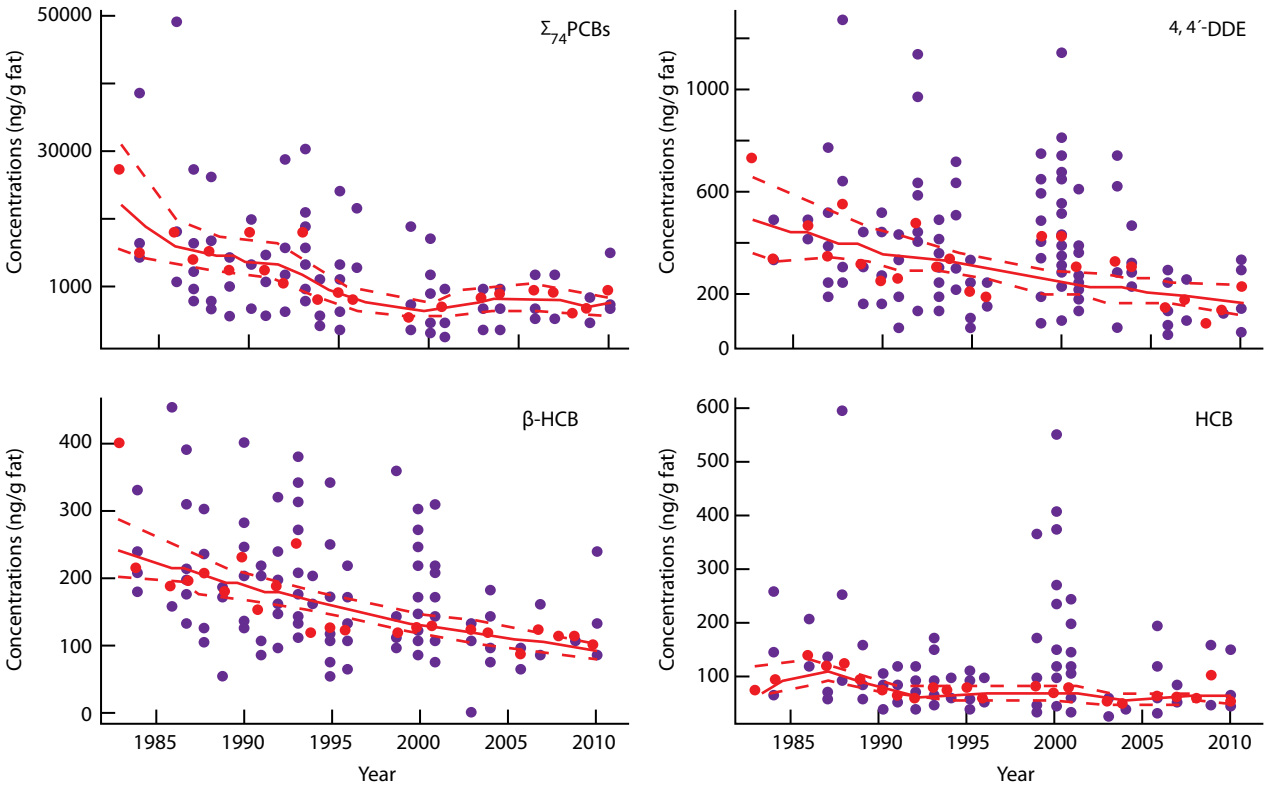


Figure 3.19. Long-term temporal trends in concentrations (ng/g fat) of PCBs (sum of 74 congeners), 4,4'-DDE, β-HCH and HCB in fat from juvenile polar bears from East Greenland. The filled red dots are median values. Red lines indicate significant trends and dotted lines the 95% confidence intervals. (based on figure from Dietz et al., 2012).

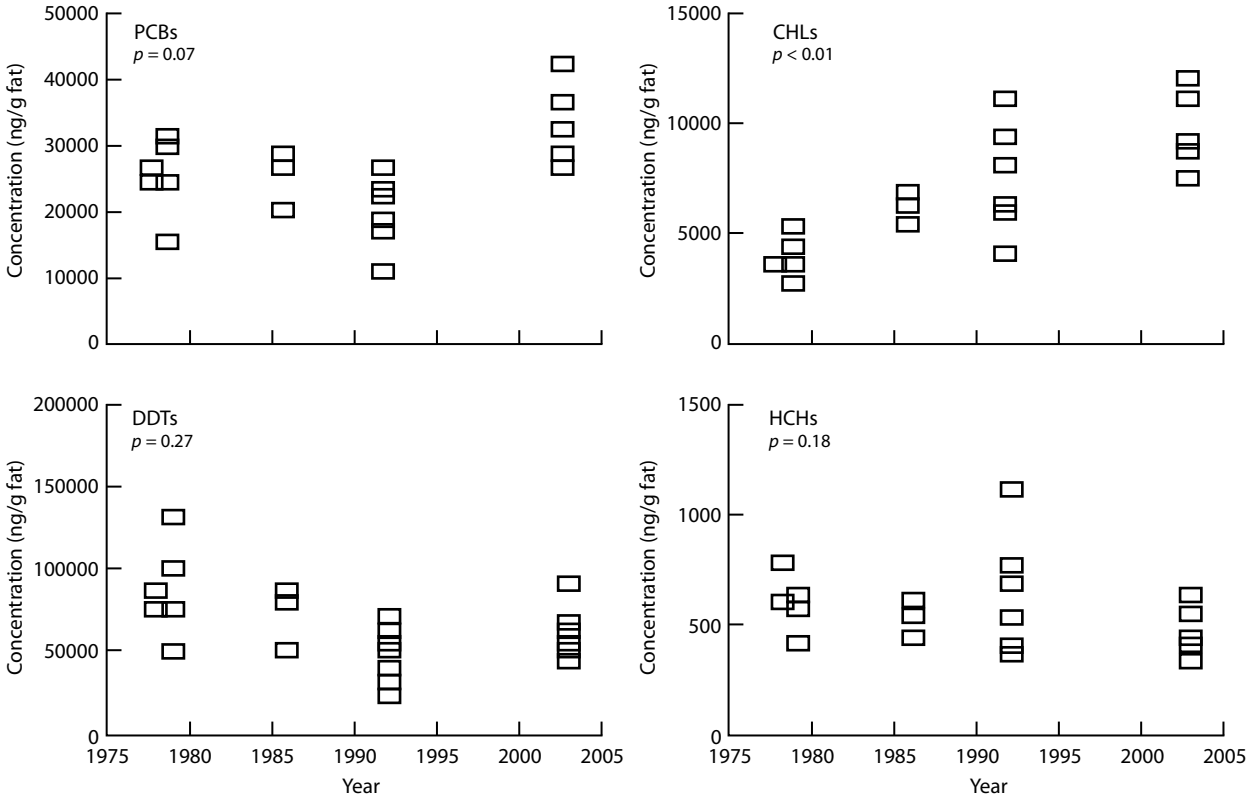


Figure 3.20. Temporal trends in concentrations (ng/g fat) of abundant organohalogen compounds in blubber of stranded striped dolphins from Japan, 1979-2003 (data from Isobe et al., 2009). PCBs = Total PCB based on technical Kanechlor mixtures; 4,4'-DDTs = 4,4'-DDT + 4,4'-DDE + 4,4'-DDD; CHLs = trans/cis-nonachlor, trans/cis-chlordane, oxychlordane; HCHs = α-, β-, γ-HCH.

(e.g. Barra et al., 2006) and Africa (e.g. UNEP, 2009a), there are no temporal trends of PCBs and organochlorine pesticides such as DDT in wildlife yet available.

PBDEs and HBCDD

Extensive time trend data are now available for PBDEs and HBCDD as a result of ongoing collections and analyses by various monitoring programmes in the Baltic, the Great Lakes, the Arctic and in Asia Pacific, as well as by retrospective analysis of samples from wildlife specimen banks. In the Baltic, the significant increasing concentrations of BDE-47 and BDE-99 and other congeners related to PentaBDE products that were found in guillemot eggs from the late sixties until the early nineties are followed by decreasing values during the period 1998-2008 (Bignert et al., 2010) (**Figure 3.21**). Similar trends for pentaBDEs were observed in a retrospective study of peregrine falcon eggs from southern Sweden (Johansson et al., 2011). In contrast HBCDD showed an increase in guillemot eggs averaging 2.9% per year from the late 1970s to 2008 (**Figure 3.21**).

No significant time trends in ΣPBDE concentrations in Indo-Pacific humpback dolphins and finless porpoises from Hong Kong Harbor were observed from 1997 to 2008 (**Figure 3.22**)(Isobe et al., 2009; Lam et al., 2009). The dolphins and porpoises have resident populations distributed in the northwestern and eastern waters of Hong Kong. ΣPBDE concentrations may have increased over the period 1997-2003 in both species, although sampling years are limited. These results were consistent with the temporal patterns of PBDEs observed in California sea lions stranded between 1994 and 2003 near San Francisco (Stapleton et al., 2006). In contrast, significantly increasing concentrations of HBCDD were found in dolphins from Hong Kong (**Figure 3.22**). This increase in HBCDD was consistent with several other studies on marine mammals from California (Stapleton et al., 2006), Japan (Isobe et al., 2009) and the UK (Law et al., 2008a).

ΣPBDEs in juvenile polar bears from East Greenland achieved maximum concentrations in 2004-05 (**Figure 3.23**) (Dietz et al., 2012), which is about 10 years later than for top predators in the Baltic (Bignert et al., 2010; Johansson et al., 2011) and in UK coastal waters (Law et al., 2010). This presumably reflects delayed exposure of the Greenland polar bears due to long-range atmospheric and oceanic transport. Similar to Baltic guillemots and marine mammals in the UK, the USA and east Asia, HBCDD was found to be increasing in polar bears, with highest median values in samples from 2010 (Dietz et al., 2012).

PFCs

Houde et al. (2011) have reviewed temporal trends of PFSAs and PFCAs in wildlife to 2010. Between 1999 and 2008, a decline of PFSAs (mainly PFOS) and PFCAs (PFNA, PFDA, PFUnA) was found in harbour seal liver samples collected in the German Bight (**Figure 3.24**). With this decline was a large

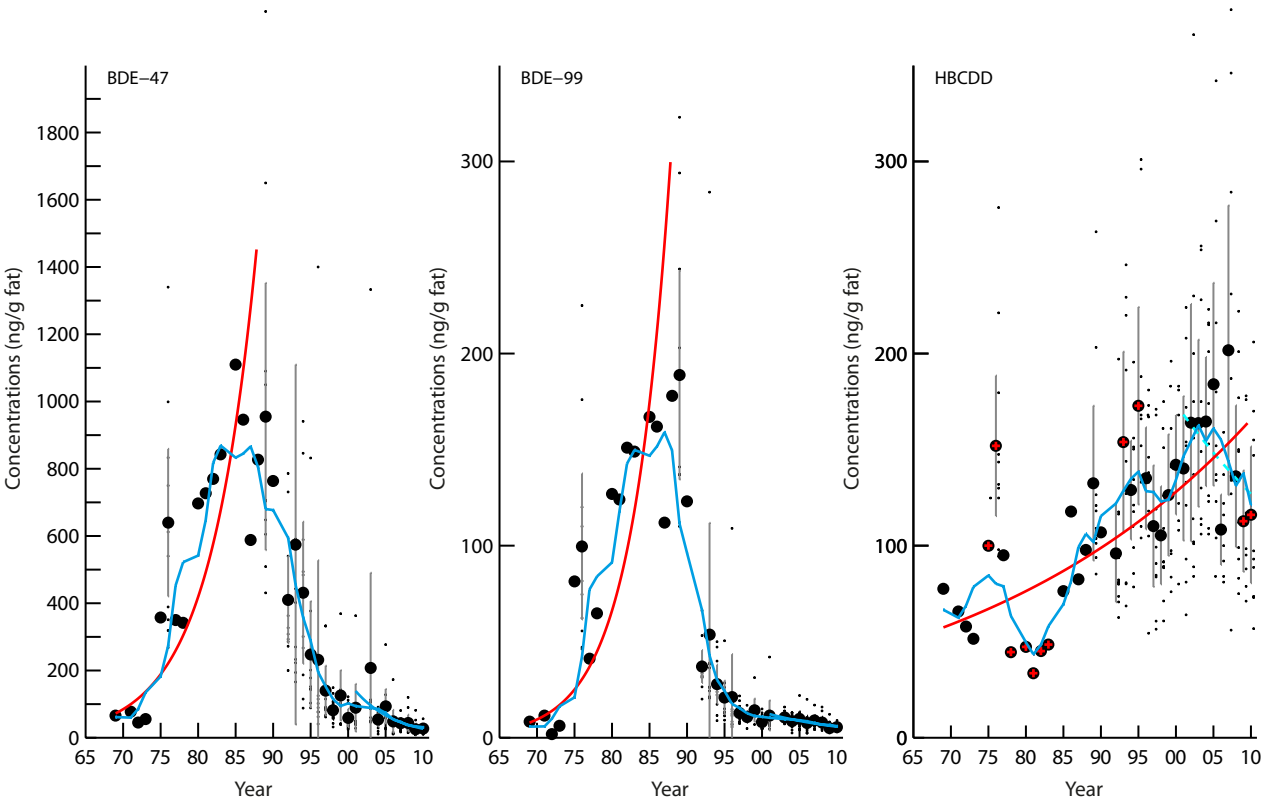


Figure 3.21. Temporal trends in concentrations (ng/g fat) of the PBDE congeners, BDE-47 and BDE-99, and of HBCDD in guillemot eggs from southern Sweden from Gotland (Baltic Sea), from 1968 to 2008 (Bignert et al., 2010).

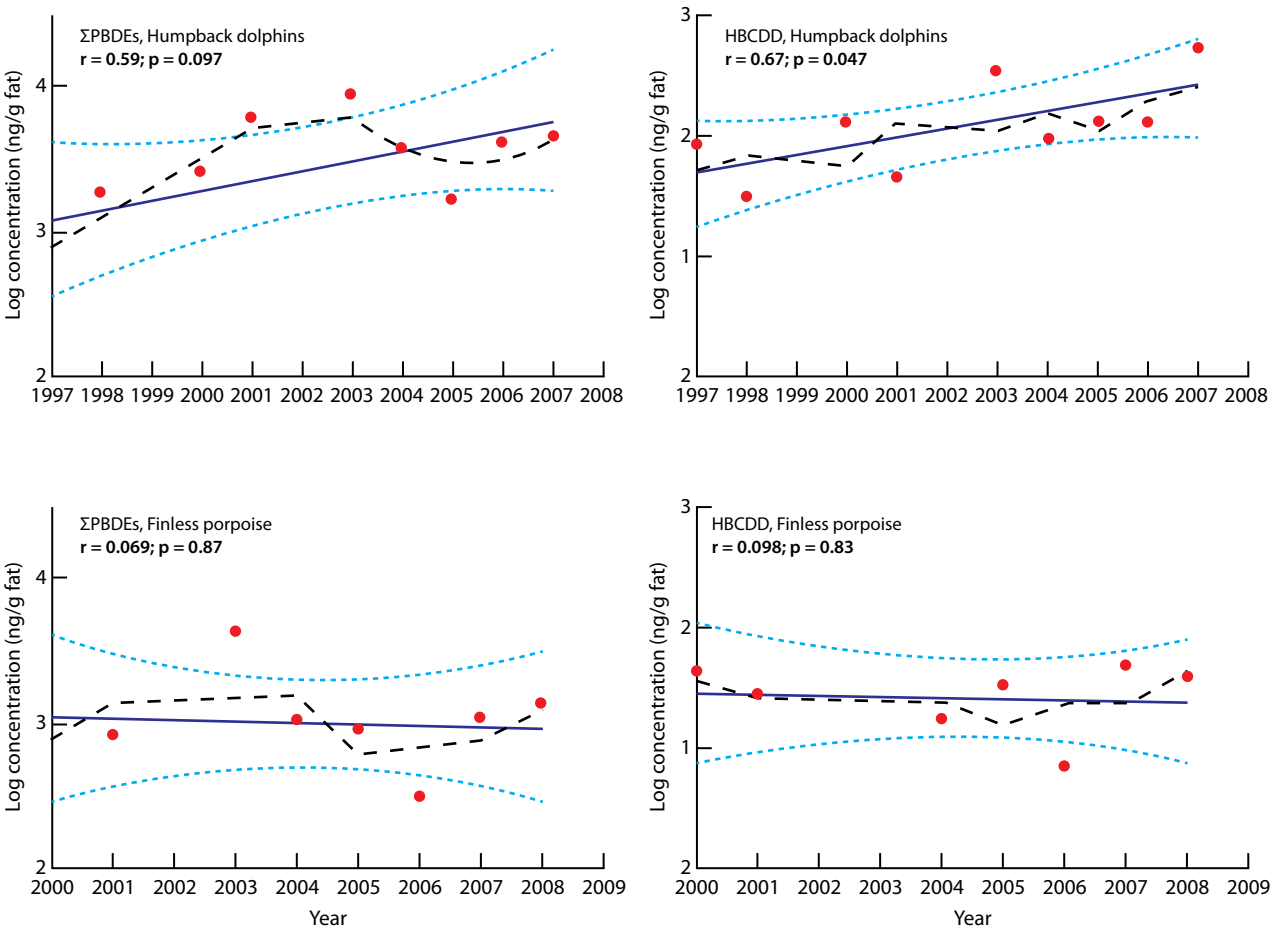


Figure 3.22. Temporal trends in concentrations (ng/g fat) of PBDEs (sum of 14 congeners) and of HBCDDs (sum of 3 congeners) in blubber of Indo-Pacific humpback dolphins (1997-2007) and finless porpoises (2000-2008) from Hong Kong Harbour. Longer dashed lines represent three-year moving averages. Shorter dashed lines represent 95% confidence intervals. (based on figure form from Lam et al., 2009).

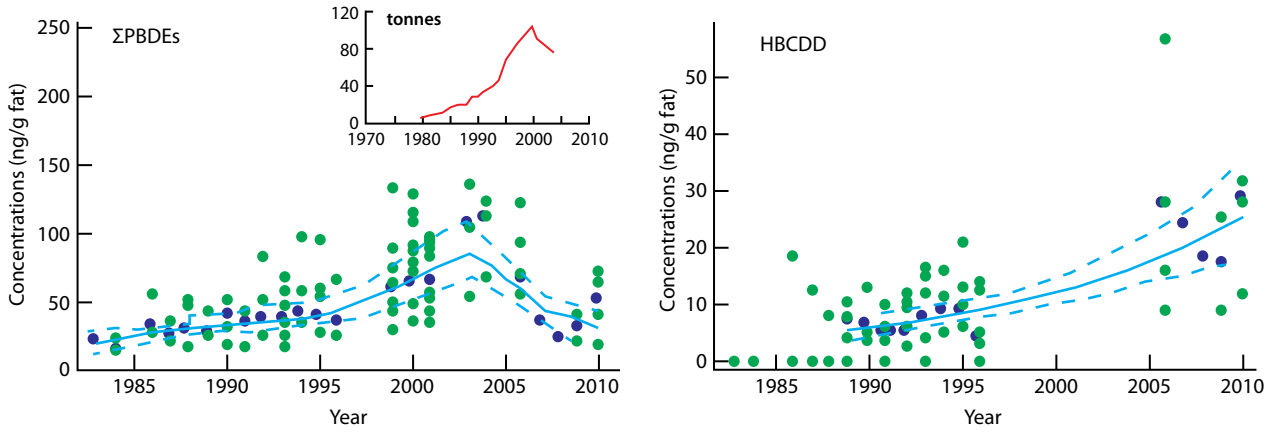


Figure 3.23. Temporal trends in concentrations (ng/g fat) of PBDEs and HBCDD in fat from juvenile polar bears from East Greenland. The filled red dots are median values, the red lines indicate significant trends, and the dotted lines show the 95% confidence intervals**.** Inset graphic: The estimated time trend of BDE-47 emissions in North America based on Alcock et al. (2003). (based on figure from Dietz et al., 2012).

decrease (~95%) in the PFOS precursor, PFOSA (Ahrens, Siebert & Ebinghous, 2009). Other marine mammals showed more variable trends, although these data are not as recent (**Figure 3.24**). Increasing concentrations of both PFSAs and PFCAs were observed in East Greenland polar bears up to 2005 and in melon-headed whale in Japan up to 2000. Northern sea otters did not show a distinctive trend in PFCs over the period 1986 to 2000 (**Figure 3.24**). Rüdel et al. (2011)

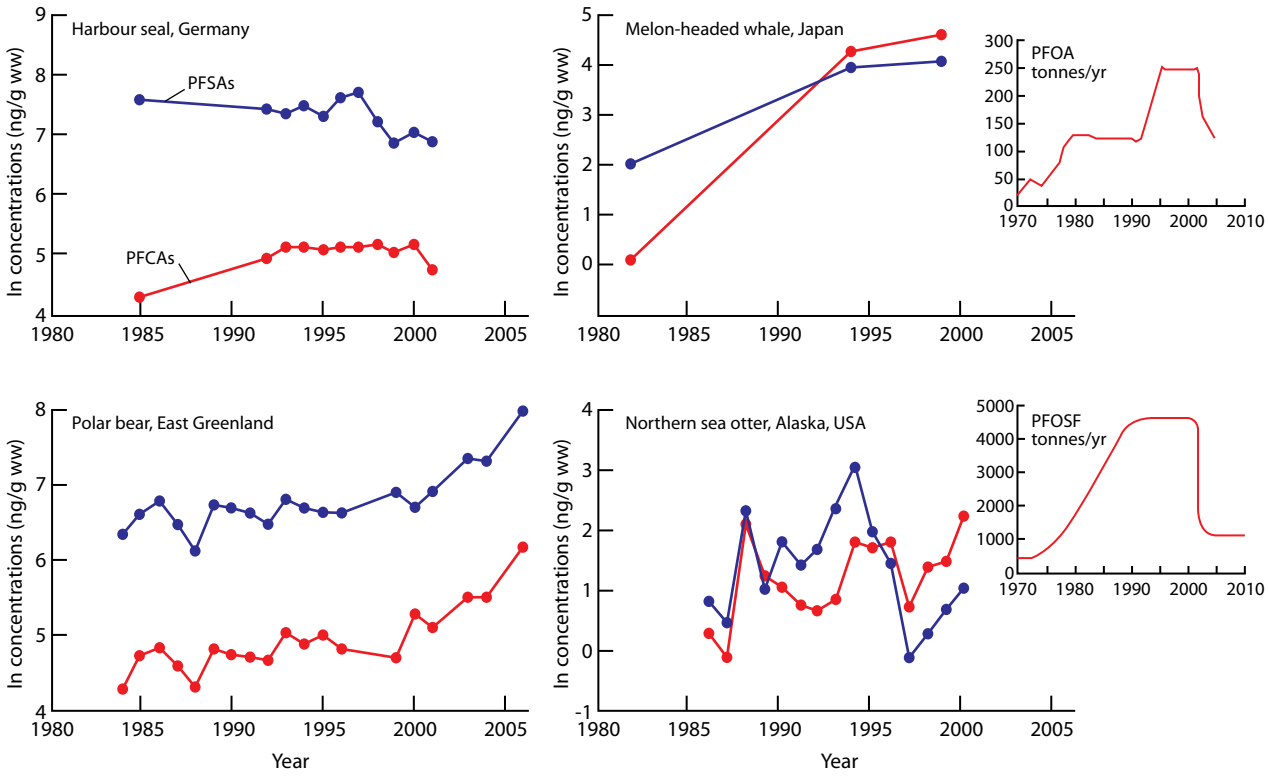


Figure 3.24. Temporal trends in concentrations (ng/g wet weight) of perfluorinated carboxylates (PFCAs) and perfluorinated sulfonates (PFSAs) in tissues of marine mammals (Houde et al., 2011). Inserted graphs: Estimated maximum global emissions of PFOA (Armitage et al., 2006) and estimated total global production of PFOSF, the major PFOS starting material (Paul, Jones & Sweetman, 2009). Composite figure based on the references given.

noted a decline of PFOSA over the period 1993 to 2008 in pooled samples of blue mussels from the sites in the North Sea and southern Baltic Sea while PFOS showed no trend. In the same region where the mussels were sampled, PFOS and PFOA in herring gull eggs showed a slow decline from maxima in the late 1990s except in the Baltic where increasing levels in eggs were observed. Bignert et al. (2011) also found highest mean concentrations of PFOS in guillemot eggs from Gotland (southern Baltic) in the late 1990s and continuing elevated levels to 2009, despite the phase out of most PFOS use in Europe as of 2001. A review of temporal trend studies of PFOS in Arctic biota show generally increasing levels of PFCs from the 1970s, although some studies from the Canadian Arctic show declines in PFOS levels from the mid-2000s (Butt et al., 2010). In general, PFOS concentrations appear to be declining in biota from sites nearer sources of PFOS and its many precursors and not in remote sites where exposure occurs via long-range ocean transport.

Mercury

Many time series for mercury in aquatic biota exist for local pollution issues and for national and regional assessments. At the regional level, there are trends for 20+ years available from biomonitoring programmes in the Baltic (Bignert et al., 2010; 2011), the North Sea (OSPAR, 2009; 2010), the Arctic (Rigét et al. 2011), and the Great Lakes (SOLEC, 2009). These programmes typically summarize trends in multiple species, e.g. Rigét et al., (2011) assessed 83 times series studies for mercury in Arctic fish, seabirds and marine mammals. No equivalent assessments of local time series studies appear to be available for East Asia, Africa or South America. In general, regional assessments have shown mercury/methylmercury trends to be variable among species within the same area; more specifically, it can be increasing in one species and declining in another. For example, mercury concentrations in guillemot eggs (Gotland, central Baltic) have decreased significantly, whereas mercury concentrations in herring from the southern Baltic were unchanged over 25+ years (**Figure 3.25**) (Bignert et al., 2011).

In the Arctic, mercury concentrations in eggs of thickbilled murres (Brünnich's guillemot) have increased about 3-fold over 35 years, while in northern fulmars the increase has been less pronounced (~50%) over the same period (Braune et al., 2010; **Figure 3.26**). In ringed seals from the same region, mercury concentrations have remained similar over this same period, although data are more limited for the 1980s (Gaden et al., 2009; Muir et al., 2011). A key factor influencing mercury trends is the production and availability of methylmercury, which is dependent upon ecosystem-specific variables and the form of the mercury loaded (Munthe et al., 2007). Gaden et al. (2009) concluded that summer environmental conditions, such as timing of the ice free season, can influence the composition of

prey (mercury exposure) available to ringed seals. Finally, Rigét et al. (2011) found no overall consistent trend for mercury across 83 time series for tissues and species from the circumpolar Arctic during the period from the early 1970s to 2007. However, they did note a west-to-east gradient in the temporal trends, with larger numbers and a higher proportion of biotic datasets in the Canadian and Greenland region of the Arctic showing significant increases than in the North Atlantic and European Arctic.

In addition, special studies incorporating museum specimens have provided insights into the long term trends of mercury and methylmercury in marine biota. Vo et al. (2011) showed that black footed albatross collected between 1880 and 2002 in the north Pacific basin exhibited a 3.8 fold increase in methylmercury levels in feathers consistent with historical global and recent regional increases in anthropogenic emissions. Dietz et al. (2011) showed that mercury has been increasing slowly in Northwest Greenland polar bear hair from preindustrial times to the present. They used hair samples dated from 1300 A.D. (n = 2), museum specimens from the period 1892-1960, and contemporary collections up to 2008. They found an increasing trend between 1892 and 2008 of ~ 1.6%/year that represented a 23- to 27-fold increase from the baseline level in 1300 A.D. Thus it appears that over the past 100 years methylmercury exposure of marine biota in the northern hemisphere has increased substantially compared to pre-industrial times (Dietz, Outridge & Hobson, 2009).

3.2.2 Humans

3.2.2.1 Internal exposure

Bioaccumulation – External exposures to EDCs for humans have been discussed above in section 3.1.5. For EDCs to have an effect though, they must be taken up into the body, and transported to sites where they interfere with endocrine system processes (Chapter 1). The tissues in the body where EDCs are found depend on the properties of the EDCs; some are

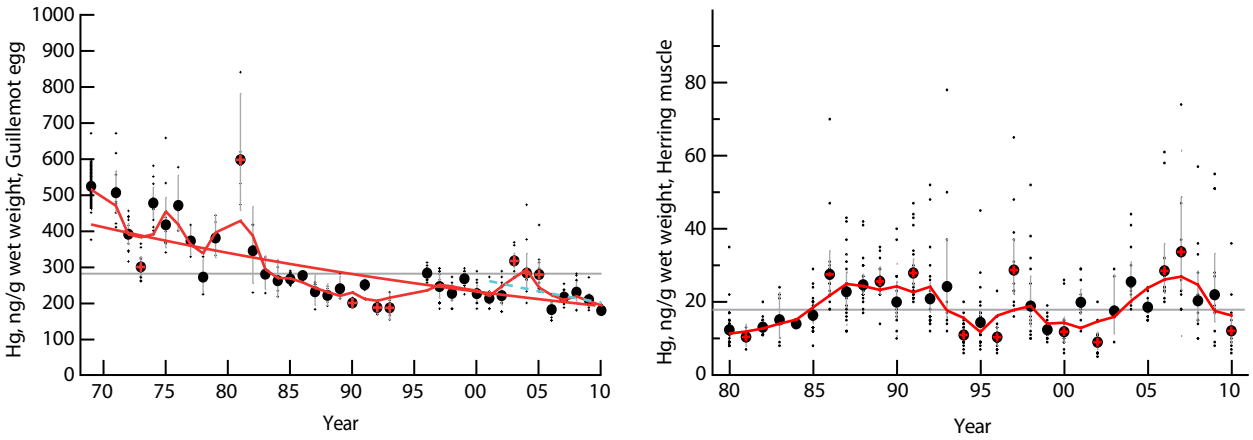


Figure 3.25. Temporal trends in concentrations (ng/g wet weight) of mercury in guillemot eggs and herring muscle from the Baltic Sea (Bignert et al. 2011).

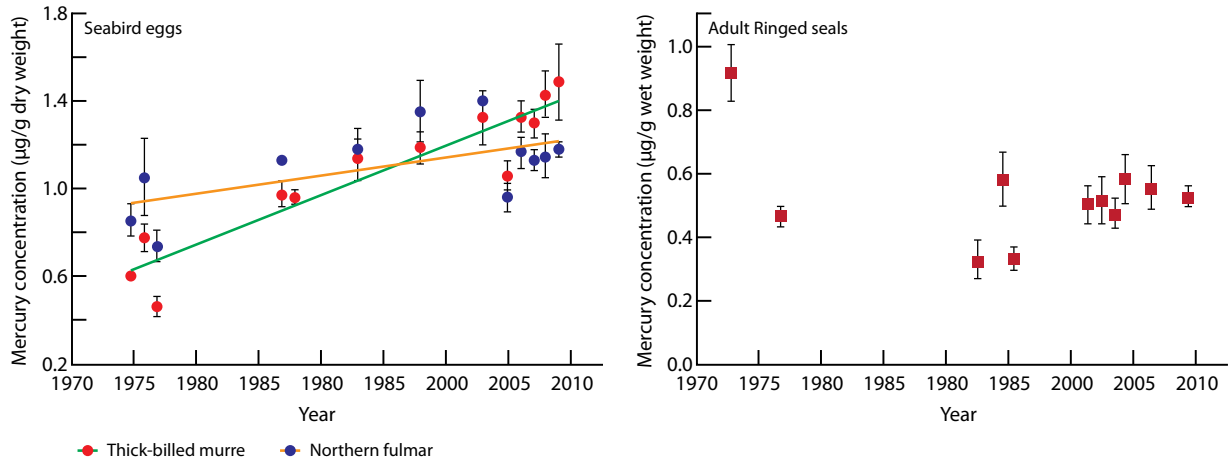


Figure 3.26. Temporal trends in concentrations of mercury in seabird eggs (µg/g dry weight; Braune et al. 2010) and ringed seal muscle (µg/g wet weight; Gaden et al. 2009; Muir et al. 2011) from the Canadian Arctic archipelago.

bioaccumulated in adipose tissue or in muscle whereas others are found in blood or tissues such as lungs or adrenals (Letcher, Klasson-Wehler & Bergman, 2000).

Animal studies have demonstrated that many EDCs are able to cross the placental barrier and thereby expose the fetus. In humans, several studies have reported detectable concentrations of EDCs in cord blood, and correlations between levels in cord blood and maternal blood (Needham et al., 2011). Transfer of chemicals between the mother and the embryo, fetus and thereafter the newborn baby is a matter of concern for humans. The similar levels of some POPs in human maternal serum, cord serum and in mothers' milk are shown in the diagram in **Figure 3.27**, emphasizing the transfer of EDCs to the fetus and the newborn nursing child (Needham et al., 2011). In contrast to the lipophilic POPs (e.g. DDTs, PCBs and PBDEs), perfluorinated alkyl acids (such as PFOS and PFOA) are proteinophilic. Concentrations of PFOS and PFOA in mothers' milk are therefore much lower than in blood (Kärrmann et al., 2007). Nevertheless, it has been demonstrated that mothers' milk is a significant exposure source for the nursing infant (Thomsen et al., 2010a) and the estimated dietary intake for a nursing infant is similar to that of an adult.

Metabolism - Metabolites of several EDCs exhibit stronger endocrine disrupting potential than their parent compounds, e.g. phenolic metabolites of PCBs and PBDEs (Dingemans et al., 2008; Hamers et al., 2008; Meerts et al., 2004). In some cases, the metabolism even leads to more persistent compounds that accumulate in tissues (Letcher, Klasson-Wehler & Bergman, 2000). Polyfluorinated telomer alcohols can be metabolized in the organism to perfluorinated carboxylic acids with half-lives of several years (Lau et al., 2007). Decabromodiphenyl ether (decaBDE), with a half-life of about two weeks in human blood (Thuresson et al., 2006), is debrominated in the body to more persistent nona- and octabrominated diphenyl ethers as experimentally shown in rats (Huwe & Smith, 2007).

Excretion - EDCs can be excreted from the body in multiple ways, and excretion rates are highly dependent on chemical properties. Nonpersistent EDCs are rapidly metabolized, primarily via the liver, and excreted through urine or faeces. More persistent EDCs can be excreted, but because they tend to accumulate in different parts of the body, such as fat, they are released much more slowly. One pathway that is unique to mothers and infants is excretion via breast milk. Persistent, lipophilic EDCs are excreted in mothers' milk, leading to the exposure of the breast fed infant (WHO, 2010c). Studies on the elimination rates of EDCs during lactation have demonstrated a significant transfer of EDCs to the breast fed child. In many cases, the exposure of the infant to POPs during breast feeding exceeds the tolerable daily intake defined for lifelong exposure (Polder et al., 2008a; 2008b). However, for optimal infant feeding, WHO recommends "exclusive breastfeeding for 6 months" (WHO, 2001). "Breastfeeding is an important source of nutrients for an infant and numerous health benefits from breastfeeding have been documented" (American Academy of Pediatrics, 2005).

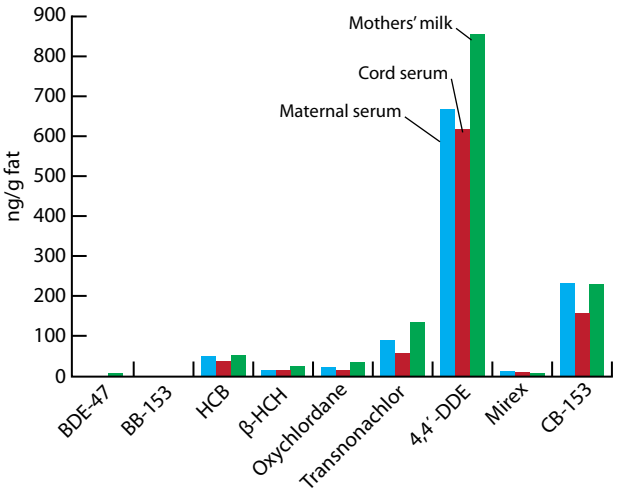


Figure 3.27. Concentrations (ng/g fat) of nine selected POPs in maternal serum, cord serum and mothers' milk from the same individual from the Faroe Islands (diagram prepared on basis of data from Needham et al., 2011). The selected abbreviated compounds are: BDE-47 = 2,2',4,4'-tetrabromodiphenyl ether; BB-153 = 2,2',4,4',5,5'-hexabromobiphenyl; HCB = hexachlorobenzene; β-HCH = β-hexachlorocyclohexane; 4,4'-DDE = 1,1-bis(4-chlorophenyl)-2-dichloroethene; CB-153 = 2,2',4,4',5,5'-hexachlorobiphenyl.

3.2.2.2 What has been measured in humans

Biomonitoring using suitable human tissues can give an integrated measure of the internal exposure from different exposure pathways. It has been widely used for monitoring exposure to persistent halogenated EDCs ever since Laug, Kunze & Pitchett (1951) detected DDT in mothers' milk from non-occupationally exposed mothers. From the 1970s and onwards, much effort was focussed on biomonitoring POPs (UNEP, 2009c; Konishi, Kuwabara & Hori, 2001; Norén & Meironyté, 2000). More recently, human biomonitoring has also been applied to more easily metabolizable compounds, such as phthalates, bisphenol A and PAHs (e.g. NHANES (USA); Swedish monitoring programme; Knudsen & Merlo, 2012).

Blood is commonly used to assess internal exposures for persistent and bioaccumulative chemicals in human populations. As the lipophilic chemicals (e.g. PCBs, PBDEs and organochlorine pesticides) are associated with blood fats, analysis is performed on serum or plasma. However, due to the low fat content of human serum and plasma, the detection can be difficult at low concentrations. Also for breast milk analyses several ml are used. The challenge with blood analysis is that sample volumes are often limited especially for biobank samples.

Several of the halogenated phenols, original compounds or metabolites of POPs accumulate in blood due to their proteinophilic properties. Both the POPs and the halogenated phenols are also efficiently transferred through the placenta (Park et al., 2008; Wan et al., 2010). The concentrations in

human blood of, e.g. OH-PCBs can reach about 20% of the PCB levels in the fats (Linderholm et al., 2007; Park et al., 2008). Further, pentachlorophenol levels in blood are in the high µg/g range on a fat weight basis (Zheng et al., 2011), which is far higher than concentrations of individual PCB congeners (Glynn et al., 2011). Perfluorinated alkyl acids (PFAAs) are likewise proteinophilic, and as little as 150 mL of serum is sufficient to measure a wide range of PFAAs (Haug, Thomsen & Becher, 2009).

Blood is also used to determine effective internal doses of non-persistent chemicals which are *per se* reactive or form reactive intermediates in humans and thereby bind to proteins or DNA. Examples are DNA adducts of BaP and haemoglobin adducts of acrylamide (Knudsen & Merlo, 2012). For non-persistent chemicals with an elimination half-life of a few hours, concentrations in blood decrease quickly after the exposure and are usually lower than those in urine (Needham & Sexton, 2000). As a result, concentrations of the chemicals in blood will be highly dependent on the time elapsed since exposure and, in many cases, below the concentration that can be measured (below the limit of detection). Determination of blood concentrations of rapidly excreted chemicals may therefore have limited value for biomonitoring purposes in epidemiological investigations.

Urine is used for human biomonitoring of compounds that are rapidly metabolized and excreted such as phthalates, bisphenol A and PAHs. When considering chemicals like these with short half-lives in the body, it is very important to regularly assess exposure since urine levels can vary over short periods and do not reflect long-term exposures (Preau et al., 2010). Spot (one time) urine samples do not give a representative picture of the internal exposure, as excretion of the chemical and urine dilution vary during a day. Corrections for dilution have been made by normalizing concentrations to creatinine content or specific weight. Twenty four hour urine samples would give the best picture of recent exposure, however this is not always feasible in large scale epidemiological studies.

While blood and urine are the most common biological matrices used for biomonitoring, many other tissues or fluids have been used, for example hair, toenails, and saliva. In addition, a number of other biological matrices are used to assess internal exposures in pregnant women and infants, including amniotic fluid, placenta, cord blood, and meconium (Koch & Calafat, 2009).

Further, mothers' milk biomonitoring has been used to determine both internal doses to the lactating woman and exposures to the developing baby. Mothers' milk biomonitoring is most amenable for measuring POPs such as organochlorine pesticides, PCBs, dioxins and PBDEs. The non-invasive sample collection and the high fat content make mothers' milk an ideal human sample for these EDCs.

Autopsies and biopsies have been performed in certain cases to obtain human tissues for analysis, but this is not feasible for large scale monitoring. For example, lipophilic POPs have been analysed in adipose tissue as this is a depot for these compounds (Guvenius, Bergman & Norén, 2001; Yu et al., 2010). The liver is a target for PBDEs and higher concentrations have been found in human liver than in adipose tissue (Doucet et al., 2009).

Availability of biomonitoring data has greatly accelerated since the 1990s, driven by both government investment and enhanced technical capabilities. The most comprehensive studies include the National Reports on Human Exposure to Environmental Chemicals conducted by the USA government, the German Environmental Survey conducted by the German Federal Government, and the Arctic Monitoring and Assessment Programme, an international programme to implement components of the Arctic Environmental Protection Strategy and administered by eight countries with land mass above the Arctic Circle (Porta et al., 2008). For example, the USA has made large investments in their national biomonitoring programme, which is administered by the Centers for Disease Control and Prevention (CDC). The programme collects blood and urine from a representative sample of the USA population as part of the National Health and Nutrition Examination Survey (NHANES) and analyses the NHANES participants' blood and urine for various chemicals (Centers for Disease Control and Prevention 2008). The number of chemicals the government has measured and reported has increased from a little over 100 to well over 200 persistent and non-persistent compounds between late 1990 and 2011 (Centers for Disease Control and Prevention 2008). Many chemicals are found ubiquitously in the USA population. Some of the chemicals found in virtually all the population include a number of POPs. Some of these chemicals have been banned in the U.S. for over 30 years (such as DDT, and PCBs), as well as in many other parts of the world. Others include persistent and bioaccumulative chemicals that have been more recently phased out. These include several PFCs (PFOS, PFOA, PFHxS, and PFNA have been found in over 98% of the USA population) and PBDEs (BDE-47, BDE-100, and BDE-153 found in over 95% of the USA population) (Calafat et al., 2007; Centers for Disease Control and Prevention, 2008). A number of less persistent compounds are also found in virtually all of the USA population including certain phthalates, PAHs, phenols, such as bisphenol A, and perchlorate (Centers for Disease Control and Prevention, 2008). While levels of lead in the USA have declined greatly, due to the removal of lead from gasoline, paint, food cans and other products, there are populations both within and outside the USA with high blood lead levels, often from exposure to lead contaminated paint (US EPA, 2010b). In addition, exposure to many compounds, such as organophosphate pesticides, is still found in relatively large segments of the population (e.g. 16 to 89% of children) (Payne-Sturges et al., 2009). Other studies have also found multiple chemicals measured in human tissues in Europe and in populations in the Arctic – indicating a global distribution of pollution exposure (Porta 2004; Porta et al., 2008).

Consequently, there is simultaneous exposure to multiple chemicals in the population. In particular, some exposures are common during sensitive periods of development. A study published by Woodruff et al. found that virtually all pregnant women in the USA are exposed to at least 43 different chemicals, though there were some chemicals that were detected in few to no pregnant women (Woodruff et al.,

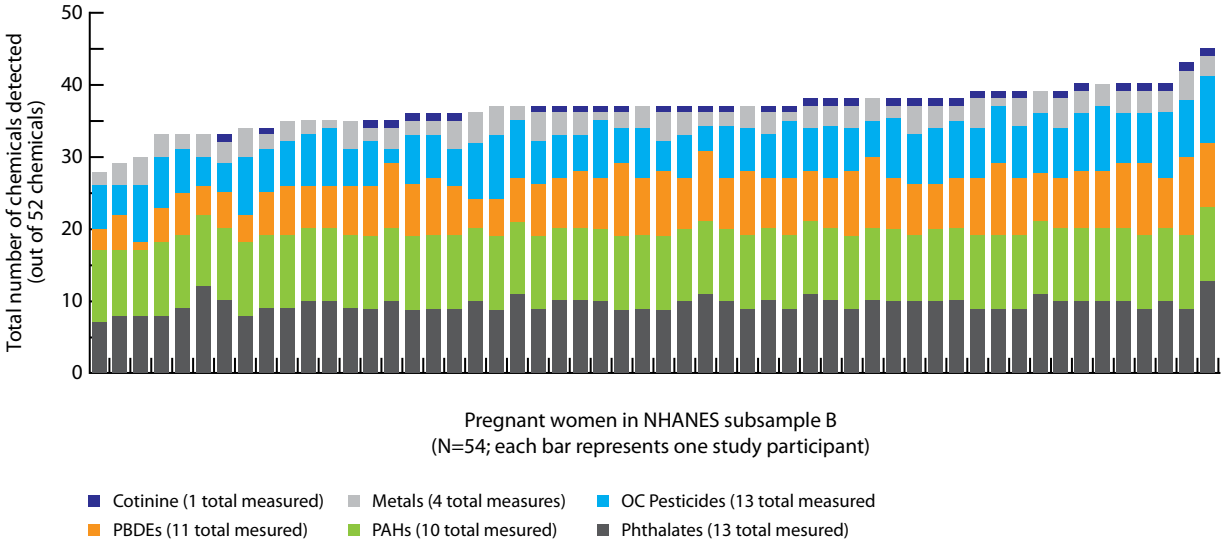


Figure 3.28. Number of chemicals detected by chemical class in pregnant women in the USA (National Health and Nutrition Examination Survey subsample B (metals, cotinine, organochlorine pesticides, phthalates, PBDEs, and PAHs), 2003-2004. (Figure from Woodruff, Zota & Schwartz (2011), redrawn; Used with publisher's permission).

2008) (**Figure 3.28**). While the authors did not evaluate the observed levels with potential adverse health consequences, many of the chemicals measured were similar to levels measured in epidemiologic studies finding an association with adverse reproductive and developmental outcomes. These include: phthalates and an increased risk of adverse male reproductive outcomes when exposure occurs prenatally (Swan et al., 2005; Chapter 2.3); mercury and developmental neurological outcomes (Lederman et al., 2008; Chapter 2.6), PBDEs and neurodevelopmental outcomes (Herbstman et al., 2009; Chapter 2.6); and PCBs and maternal thyroid hormone disruption during pregnancy (National Toxicology Program, 2006; Chevrier et al., 2008; Chapter 2.5 & 2.6).

Exposures can also be higher in certain populations, including children, and in certain types of exposure situations such as occupational settings. For example, women living in the agricultural Salinas Valley of California had higher measurable levels of several pesticides compared to a representative sample of pregnant women in the USA (Castorina et al., 2010). Children can have higher exposure because of unique behaviours, i.e. smaller children have more exploratory behaviour, and put their hands in their mouth more often, which can lead to increased exposure to chemicals that are prevalent in objects they come in contact with, such as toys or dust (US EPA, 2008). For example, limited data on PFCs found higher levels in children ages 3 to 11 years when compared with other age groups (Kato et al., 2009; Toms et al., 2009). Another example is for PBDEs (e.g. hand-to-mouth behaviour or particular diets); these data also indicate that children younger than 7 can have the highest exposures. A large study conducted in Australia found that the levels of PBDEs in blood were greatest for children ages 2 to 6 years, compared with older children and adults (Toms et al., 2009). A study of 20 young children (ages 1.5 to 4 years) in various locations throughout the USA found that their PBDE blood levels were about 3 times higher than those of their mother (Lunder et al., 2010). In California, children 2 to 5 years of age had PBDE blood levels that were greater than those in adults and in children of a similar age in other parts of the USA; this difference is likely due to California's unique regulatory requirement for foam to meet certain standards for flammability that require the use of chemicals, and the ubiquitous presence of PBDEs in the house, particularly house dust (Zota et al., 2008; Rose et al., 2010).

Exposures to multiple chemicals has implications for assessing risks, as studies find that exposures to several chemicals that adversely affect the same common health outcome can result in a greater risk than exposure to an individual chemical (National Research Council, 2008). The USA National Academy of Sciences recommends that health risk assessments incorporate simultaneous exposures to multiple chemicals (National Research Council, 2008).

3.2.2.3 Spatial trends for humans

PBDEs

Biomonitoring of PBDEs has been used to identify geographic differences in exposures. On a global scale, the USA population has higher PBDE body burdens than people in Europe or Asia (Toms et al., 2011; **Figure 3.29)**, except for those regions of developing countries where people have been exposed to, e.g. e-waste. Blood levels of the dominant congener BDE-47 in people in North America (0.63-46 ng/g fat) are approximately one order of magnitude higher than those observed in Europe (0.24-2.4 ng/g fat) (Fredriksen et al., 2009), likely due to higher use of PentaBDE, OctaBDE and DecaBDE in North America. Zota et al. (2008) found that serum levels of PBDEs were about two times higher in California compared to

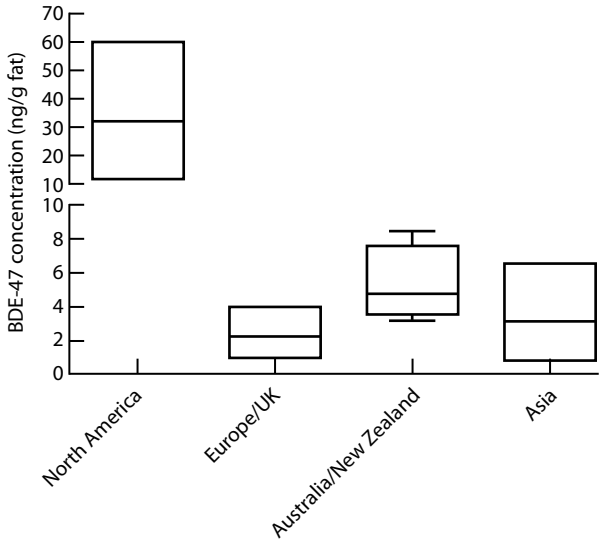


Figure 3.29. Range (min. to max.) of BDE-47 concentrations (ng/g fat) in human serum by continent (Toms et al., 2011). (Figure redrawn; Used with publisher's permission).

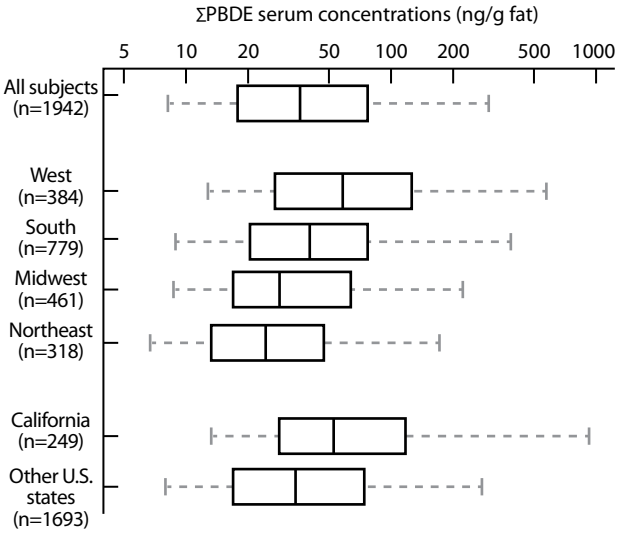


Figure 3.30. Differences in PBDE serum concentrations (ng/g fat) by geographic region within the USA using data from the 2003-2004 National Health and Nutrition Examination Survey. (Figure from Zota et al. (2008), redrawn; Used with publisher's permission).

the rest of the USA (**Figure 3.30**). Further, a small study of low income pregnant women in California found the highest levels of PBDEs measured in pregnant women in the world (Zota et al., 2011). When compared to similar-aged children in other countries or parts of the USA, children in California (2-5 years of age) had PBDE concentrations that were 5-1000 times higher and were related to the presence of new furniture or mattresses in the home and their dietary habits (Rose et al., 2010). Other subpopulations at risk from PBDEs also include those that have unique exposure sources. For example, Thomsen et al. (2008) found significant associations between the serum levels of PBDEs in hobby anglers and the self-reported consumption of trout and pike from a BFR contaminated lake in Norway. Among the hobby anglers, the median for the sum of seven PBDEs was 18 and 8.4 ng/g fat for men and women, respectively. In the reference group eating only food with background contamination, the corresponding median was 3.7 ng/g fat for both men and women.

Phthalates

Measurement of urinary phthalate metabolites is used to estimate internal exposure to these chemicals. Most biomonitoring data on phthalate exposure have been collected for the German and the USA populations. In general, the data from both countries are in good accordance, with the highest levels found for metabolites of diethyl phthalate, DBP and DEHP (Wittasek et al., 2011). However some differences were observed; the metabolites monobutyl phthalates (MnBP and MiBP) were highest in Germany whereas the concentrations of monoethyl phthalates and monobenzyl phthalate were highest in the USA. Different patterns of phthalate use, e.g. in personal care products, may explain these different exposure levels. Data from other countries are scarce but emerging over the last years. In 36 Japanese volunteers urinary concentrations of DEHP metabolites were comparable to concentrations from the German studies (Itoh, Yoshida & Masunaga, 2007). The daily exposure back-calculated from the urinary metabolite levels showed for DEHP medians of 0.9 µg/kg body weight (bw)/ per day for the USA studies (n=6), 4.1 µg/kg bw/per day for the German studies (n=6), and 1.8 µg/kg bw/per day for the Japanese studies (n=2).

3.2.2.4 Temporal trends for humans

DEHP and DiNP

As an example of how exposure to phthalates follows the production volumes, results from investigations using the German Environmental Specimen Bank for Human Tissues are presented. Wittasek et al. (2007) measured DEHP metabolites in 24h urine samples collected from a total of 634 university students between 1988 and 2003. The calculated daily intakes of DEHP varied between 0.19 and 39.8 µg/kg body weight but showed a significant decrease over the 15 year time period from

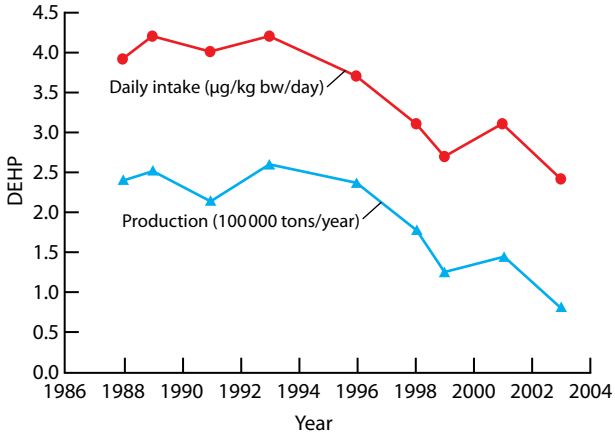


Figure 3.31. Time course of industrial DEHP production in Germany and median daily intake of DEHP in university students. (Figure from Helm (2007), redrawn; Used with publisher's permission).

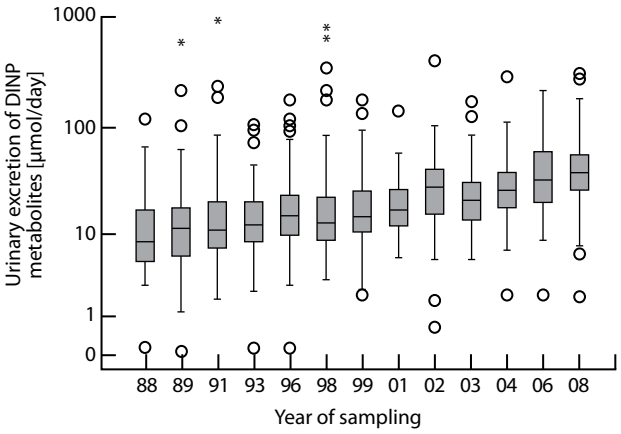


Figure 3.32. Trend of daily urinary excretion (µmol/day) of diisononylphthalete ester (DiNP) metabolites, presented as the sum of 7-hydroxy-monoisononyl phthalate ester (OH–MiNP) and 7-oxo -monoisononyl phthalate ester (oxo-MiNP), for the period 1988–2008 shown as box plots with median and central fifty percentile range. (Figure from Göen et al. (2011), redrawn; Used with publisher's permission).

a median of 4.2 µg/kg body weight/day in 1998 to 2.4 µg/kg body weight/day in 2003. Helm (2007) compared these intake data with the production data for DEHP in Germany and found a nearly perfect correlation (**Figure 3.31**). In contrast to DEHP, a continuous increase in the urinary excretion of metabolites of di-iso-nonyl phthalate (DiNP) was observed (**Figure 3.32**), starting from 10 µmol/day in 1988 to 40 µmol/day in 2008 (Göen et al., 2011). These temporal trends are explained by the restriction in use of DEHP for several applications and the substitution with DiNP.

Persistent organic pollutants

To investigate the temporal trends in human exposures to POPs, retrospective time trend studies have been performed in Norway (Haug, Thomsen & Becher 2009; Thomsen, Liane & Becher, 2007). The studies determined PCBs, PBDEs and PFCs in archived serum samples (from men 40-50 years old), pooled according to year of collection, from more than 25 years in the period 1976 up to the early or mid-2000s. For PCBs, blood levels continuously decreased over that time, corresponding to the 1980 ban on new uses of PCBs in Norway. In the same period, blood levels of PBDEs were increasing until around 2000, after which they seem to reach a plateau and possibly decrease. This may be related to voluntary actions among downstream users of PBDEs and, subsequently, the phase-out of PentaBDE and OctaBDE, due to EU legislative measures (Cox & Efthymiou, 2003). Similarly, for PFOS, a nine-fold increase in the serum concentrations of this PFC was observed from 1977 until the mid-1990s, when the concentrations reached a plateau before starting to decrease around year 2000 (see **Figure 3.33**). This may be related to the voluntary phase-out of perfluorooctylsulfonyl chemicals by the main manufacturer at that time.

Similar trends in POPs concentrations have been found in Germany. Each year since 1985 the German Environmental Specimen Bank for Human Tissues has collected and stored human specimens (mainly urine, blood and scalp hair) from about 500 volunteers at four universities in Germany. Temporal trends in concentrations of inorganic elements and a wide range of organic pollutants have been measured

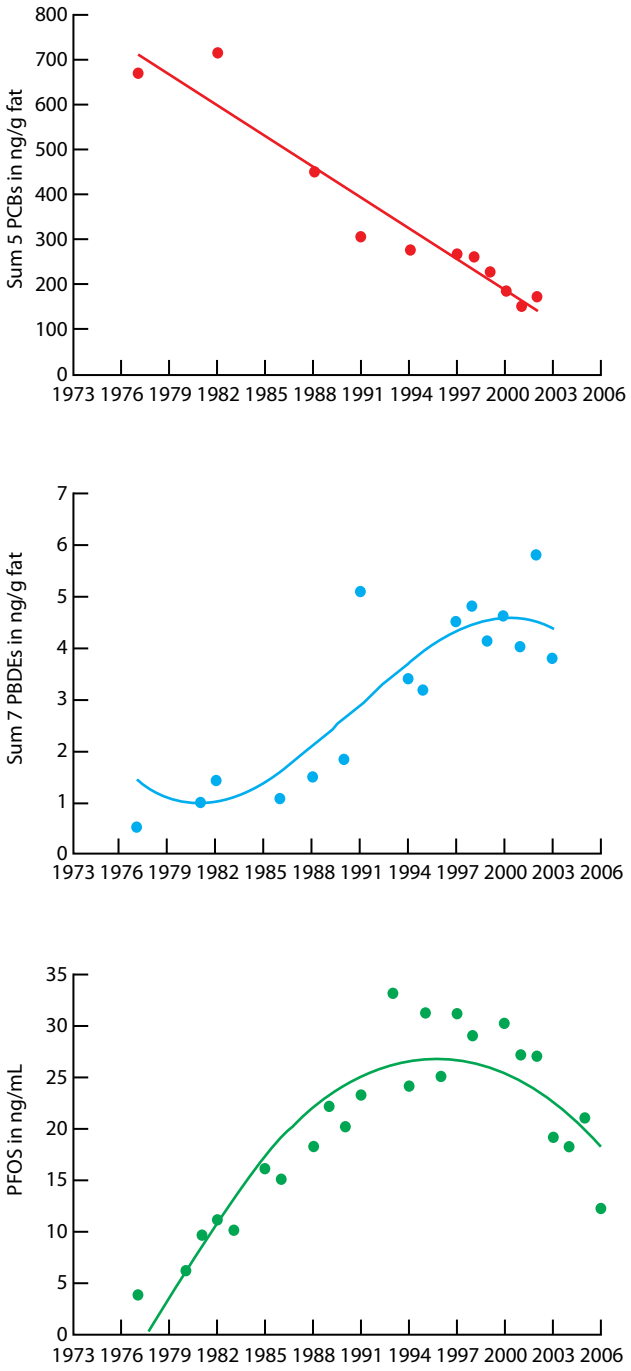


Figure 3.33. Temporal trends in concentrations of PCBs (sum of five congeners, ng/g fat; top graph), PBDEs (sum of seven congeners, ng/g fat; middle graph), and PFOS (ng/mL; bottom graph) in pooled (n>20) serum samples from Norwegian men. PCB, PBDE and PFOS concentrations were measured in the same group of men (Thomsen, Liane & Becher, 2007; Haug, Thomsen & Becher, 2009).

either in real-time or retrospectively (Wiesmüller & Gies, 2011). Plasma concentrations of chlorinated pollutants, such as pentachlorophenol, HCB, PCDDs/PCDFs and PCBs, are clearly declining in the time periods investigated, while they are increasing for PBDEs (until 1999). These trends seem to reflect the changes in production and usage patterns.

3.2.2.5 Prenatal exposure

It is well recognized that transfer of metals and xenobiotic chemicals, including EDCs, from mother to child occurs through the placenta during pregnancy (Tan, Meiller & Mahaffey, 2009; Winneke 2011; Barr, Bishop & Needham, 2007). Such in utero exposures have become an important public health concern because of the possible impact of EDCs on sensitive development and programming of organ function (Grandjean et al., 2008; see Chapter 2, all sections). Many current studies are focusing on the association between in utero exposures to a variety of environmental chemicals, in particular EDCs, and birth, developmental and neurocognitive outcomes (Herbstman et al., 2009; Suzuki et al., 2010; Tan, Meiller & Mahaffey, 2009; Chapter 2.6). The concept of "developmental origins of health and adult disease" hypothesizes that fetal and early life exposures can induce adverse effects in adulthood (Newbold et al., 2008; Fox et al., 2012).

Assessment of fetal exposures to EDCs can typically be achieved by measuring chemicals in maternal tissues as a surrogate for fetal exposure, or by measuring chemicals in cord blood, amniotic fluid or neonatal meconium (Barr, Wang & Needham, 2005). Fetal exposure to POPs and bioaccumulative metals can be assessed by making maternal blood measurements. For the non-persistent chemicals, i.e. chemicals with short environmental and biological half-lives, a single maternal measure likely does not accurately reflect total fetal exposure during gestation (Barr, Wang & Needham, 2005). Further, some EDCs can bioaccumulate in the fetus resulting in greater exposure during this very sensitive period of development. For example, fetal methylmercury levels have been shown to be about 1.7 times greater than maternal levels (Stern & S Smith, 2003). Other chemicals may not accumulate in the fetus; for example PBDEs are lower in umbilical cord blood than in maternal blood (Frederiksen et al., 2010b).

The transfer of chemicals across the placenta has also been investigated in ex vivo human placenta perfusion systems. Frederiksen et al. (2010a) studied the kinetics and the extent of placental transfer of BDE-47, -99 and -209 by adding these PBDEs to maternal circulation and monitoring the chemicals in the maternal and fetal compartments. Placental transfer was dependent on the degree of bromination. The transport of BDE-47 occurred much faster and to a greater extent than for BDE-99, while the transport of BDE-209 seemed to be very limited. Using the same approach, Balakrishnan et al. (2010) investigated the placental transfer of the non-persistent bisphenol A at environmentally relevant concentrations. About 27% of bisphenol A was detected in the fetal compartment within 3 hours, demonstrating that low concentrations can cross the human placenta, mainly in its original, unconjugated form.

Umbilical cord blood has often been used for assessing the exposure to a variety of halogenated POPs and metals. It has the advantage of being a non-invasive sample, but the sample amount is limited and the fat content is lower than in maternal blood. Thus, the detection of low levels of lipophilic POPs can be difficult (Barr, Wang & Needham, 2005). A comprehensive review of concentrations of xenobiotic chemicals in the maternal-fetal compartments has been presented by Barr, Bishop & Needham (2007). In the following, only a few examples are presented to demonstrate the pervasiveness of fetal exposures to a mixture of environmental chemicals.

Needham et al. (2011) measured the concentrations of 87 environmental chemicals in paired mother-child samples (cord serum and tissue, placenta, maternal serum and mothers' milk) from a birth cohort on the Faroe Islands, where exposures to marine contaminants is high. Virtually all substances found in mothers were also present in fetal tissues and cord blood, demonstrating that transplacental passage had occurred. For organohalogen compounds detectable in all tissues, a high correlation between concentrations in maternal serum and the other tissues investigated was generally observed. Concentrations of chlorinated POPs in cord serum were 20% of what was found in the mothers' sera; after adjusting for differences in the fat content of the sera, cord blood had slightly more than half of the POPs levels of maternal serum. In addition, mercury levels showed excellent correlations among the different sample types, suggesting that all of the tissues, including the easily collected umbilical cord, are useful for biomonitoring fetal exposure to this metal (Grandjean et al., 2005).

Park et al. (2008) investigated in detail the placental transfer of PCBs and their hydroxylated metabolites in a birth cohort from a PCB contaminated area. It was demonstrated that PCBs were transferred on a 1:1 basis between the fat compartments in maternal and fetal blood, but concentrations on fresh weight basis were lower due to the lower fat content of the fetal serum. Hydroxylated PCBs, in contrast, were not associated with fats but instead were bound to serum proteins. Thus, concentrations of hydroxylated PCBs in cord serum were quite similar to those in maternal serum on fresh weight basis. This suggests either a higher placental transfer rate of the hydroxylated PCBs compared to the original PCB compounds or a higher metabolism of PCBs in the fetus.

Frederiksen et al. (2010b) determined PBDEs in 51 pairs of maternal and cord plasma from a cohort of Copenhagen women. The concentrations observed in maternal (median 1.77 ng/g fat) and fetal (median 0.96 ng/g fat) plasma were highly correlated with each other, but the placental transport was found to decrease with increasing degree of PBDE bromination. Furthermore, positive correlations were found for the sum of PBDEs in cord blood and house dust indicating that house dust is a significant source of human exposure, including *in utero*, to PBDEs in Denmark.

Gützkow et al. (2011) determined PFAAs in 123 paired samples of maternal and fetal plasma from a subcohort of the Norwegian Mother and Child Cohort Study. Strong and highly significant correlations were found between maternal and cord blood concentrations on fresh weight basis for PFOA, PFOS and three other PFAAs. Compared to the maternal samples, cord plasma had a 1.4-4 fold lower median concentration of all the PFAAs measured. Placental transfer was found to be selective, with a higher proportion of shorter chained PFAAs in cord blood together with a higher amount of branched isomers of PFOS, indicating specific fetal exposures to some EDCs.

3.2.2.6 Case study of post-natal exposure - POPs in mothers' milk

Mothers' milk is an excellent matrix for the analysis of fat soluble pollutants, i.e. POPs and other persistent and bioaccumulative chemicals. Several reviews have addressed the issue over the last decade (e.g. Fuerst 2006; LaKind, Amina & Berlin, 2004; Norén & Meironyté, 2000; Solomon and Weiss, 2002; Tanabe & Kunisue, 2006). It is, however, possible to use mothers' milk to trace other chemicals (such as PFOS and PFOA) that have primary accumulation in blood and liver, and to assess their time trends (as shown in **Figure 3.34;** Sundström et al., (2011) and those of pentachlorophenol (Norén & Meironyté, 2000). The increasing concentrations of PFOS and PFOA stopped around year 2000 and then declined in the most recent years, likely due to legislative measures and changes in production of these chemicals (3M Company, 2000; US EPA, 2006b). For other chemicals with shorter half-lives, mothers' milk would not be an appropriate tissue to monitor for exposure assessments.

The general affinity of chlorinated and brominated POPs for fats has led to the identification of 22 POPs, HBCDD and chlorinated paraffins in mothers' milk. For some of these the dataset is very limited, e.g. chlorinated paraffins were reported in mothers' milk in Germany for the first time in 2005 (Reth et al., 2005). The year after, levels of both short- and medium-chained chlorinated paraffins (SCCPs and MCCPs) were reported for UK mothers' milk, the former in sum concentrations between 50 and 800 ng/g fat and the latter at lower concentrations (6-300 ng/g fat) (Thomas et al., 2006). The data indicate that it is primarily chain lengths of C10-C14 of the chlorinated paraffins that accumulate in the milk fat.

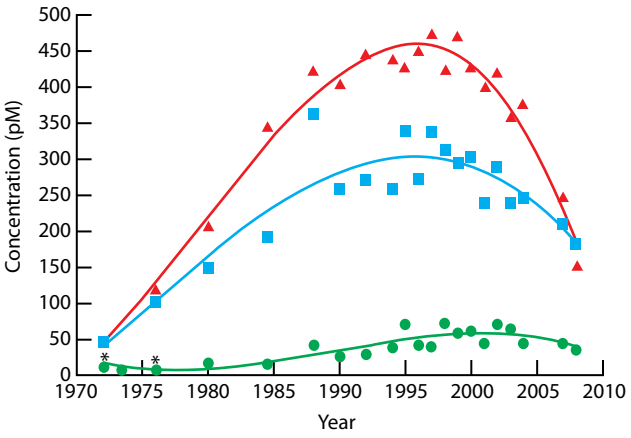


Figure 3.34. Temporal trends of PFOS (▲), PFOA (■) and PFHxS (●) (pmol) in mothers' milk from Stockholm, Sweden, from 1972 -2008 (Sundström et al., 2011).

For many of EDCs, the global coverage of their concentrations in mothers' milk is poor. For example, only a few reports from Europe and one from Canada have been published for pentachlorobenzene, showing median levels of 1 ng/g fat or less. Median toxaphene levels are higher and in the range of 10-60 ng/g fat. Some typical concentrations of the least studied POPs in mothers' milk are shown in **Table 3.2**. The WHO human milk survey included several POPs in their analysis and results from one country in each of South America, Africa and Asia are shown in **Figure 3.35** (UNEP, 2009c). Levels of ΣDDTs dominated across all of the countries.

Data on PBDEs, PFOS and HCHs in mothers' milk exist from around the world. Two of the chemicals were either just emerging or not considered 10 years ago, (i.e. PBDEs and PFOS, respectively), while HCHs were reported but had only limited data available for assessing human exposure and transfer to nursing children. Still, there are some countries from which no data have been reported. The longest temporal trend study on POPs (BDE-47, BDE-153 and HBCDD) in mothers' milk comes from Stockholm (Bergman et al., 2010; Fängström et al., 2008) and results are shown in **Figures 3.36** and **3.37**. In addition, BDE-47, BDE-153, β-HCH and γ-HCH (Lindane) levels in mothers' milk show some variability across the globe (**Figures 3.38** and **3.39**). It is important to note that almost no data were obtained from South America, while other continents are well represented.

Data on POPs in mothers' milk are dominated by reports on DDT (including DDE) and PCBs, with more than 50 reports on DDE from 1995 until today. Similarly, PCB reports number more than 100 during this period. However, it is not possible to compare all the data because different congeners were measured or the results were calculated and presented differently from one study to another (i.e. concentrations can be given in fresh weight or fat weight for one or several congeners and either as mean or median concentrations). This is a problem for the POPs most frequently reported in mothers' milk, but also occurs for any other human or wildlife tissue.

Temporal trends of 4,4'-DDE and CB-153 in Swedish mothers' milk are shown in **Figures 3.40** and **3.41**, respectively, from 1972 until 2010 (Bergman et al., 2010). Comparing these data to international levels indicate that 4,4'-DDE concentrations may be from 50 up to more than 10,000 ng/g fat in some mothers' milk, with high concentrations from Zimbabwe (Chikuni et al., 1997), India (Devanathan et al., 2009), and Vietnam (Haraguchi et al., 2009). Levels of 4,4'-DDE are more commonly between 50–1000 ng/g fat. For comparison, PCB levels (as mirrored by CB-153) range over two orders of magnitude 5–500 ng/g fat, with the lowest and highest levels in Vietnam (Tue et al., 2010a; 2010b; Nguyen

Table 3.2. Examples of mean or median concentrations (ng/g fat) for the least well studied POPs in mothers' milk worldwide. The countries from where the samples originate are given in the table, with references as footnotes.

Country	Heptachlor (mean)	Mirex (median)	∑PBB[8]* (mean)	PCBz (median)	Toxaphene (mean)	HBCDD (mean)
Australia		0.2[1]				
Canada		2[2]		1[2]	7[2]	
China					1[3]	
Denmark		0.2[4]	0.3[4]	0.3[4]		
Finland		0.3[4]	0.2[4]	0.2[4]		
Germany	20[5]					
Japan						1[6]
Jordan	500[7]					
Mexico	600[8]			200[8]		
Norway		0.6[9]				2[10]
Russia		0.5[11]			10[11]	0.5[11]
Spain						5[12]
Sweden						0.4[13]
Taiwan	3[14]					
The Philippines						0.9[15]
USA		2[16]				
USA		1[17]				

*BB-31, -49, -52, -77, -80, -101, -153 and -155.

[1] Mueller et al., 2008, [2] Newsome & Ryan, 1999, [3] Hedley et al., 2010, [4] Shen et al., 2008, [5] Schlaud et al., 1995, [6] Kakimoto et al., 2008, [7] Nasir, 1998, [8] Rodas-Ortíz, 2008, [9] Polder et al., 2008a, [10] Thomsen, 2010b, [11] Polder et al., 2008b, [12] Eljarrat et al., 2009, [13] Fängström et al., 2008, [14] Chao et al., 2006, [15] Malarvannan et al., 2009, [16] Greizerstein et al., 1999, [17] Madden & Makarewicz, 1996.

et al., 2010) and the Czech Republic (Cerná et al., 2010), respectively.

PCDDs/Fs in mothers' milk have been recently and extensively studied through the WHO human monitoring programme (UNEP, 2011b). The levels in mothers' milk vary between a few to almost 25 pg WHO-PCDD/F-TEQ/g fat in the 34 countries from which milk was obtained. A snapshot of TEQs (PCDD/F and DL-PCBs) in a few Eastern Asian countries is shown in **Figure 3.42** (Zheng et al., 2008a).

3.2.2.7 HPCs and Non-HPCs in mothers' milk

Whereas the contamination of mothers' milk by POPs is well documented, data on the presence of other EDCs are scarce. Ye et al. (2006) reported the presence of free and total (free plus those conjugated to endogenous molecules such as glucuronic acid or sulfate) selected environmental phenols in 20 mothers' milk samples. Bisphenol A was found at median concentrations of 0.4 ng/mL and 1.1 ng/mL for free and total species, respectively, indicating that the conjugated forms of

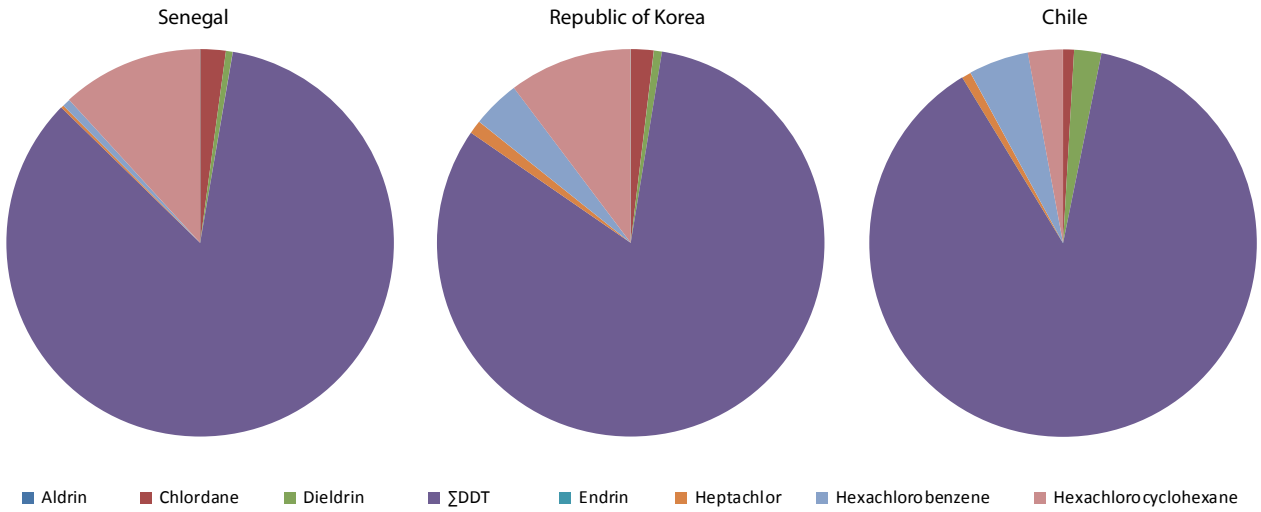


Figure 3.35. Relative content of different organochlorine pesticides in mothers' milk from three countries in three continents around the globe (diagram prepared on basis of UNEP, 2009c).

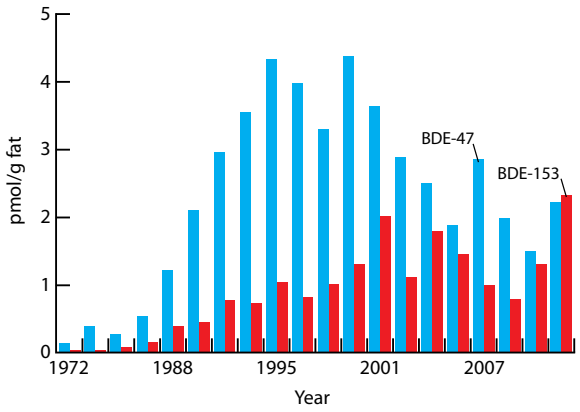


Figure 3.36. Temporal trends of the concentrations (pmol/g fat) of two PBDE congeners, BDE-47 and BDE-153, in mothers' milk from Stockholm, Sweden, as assessed 1972- 2010 (Norén and Meironyté, 2000; Bergman et al., 2010).

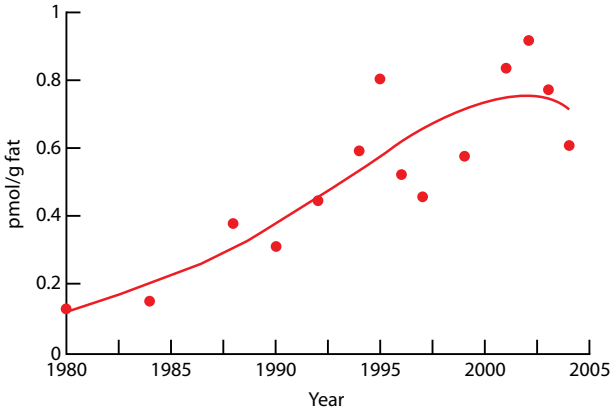


Figure 3.37. Temporal trends of HBCDD concentrations (pmol/g fat) in mothers' milk in Stockholm, Sweden, from 1980 – 2004 (Fängström et al., 2008).

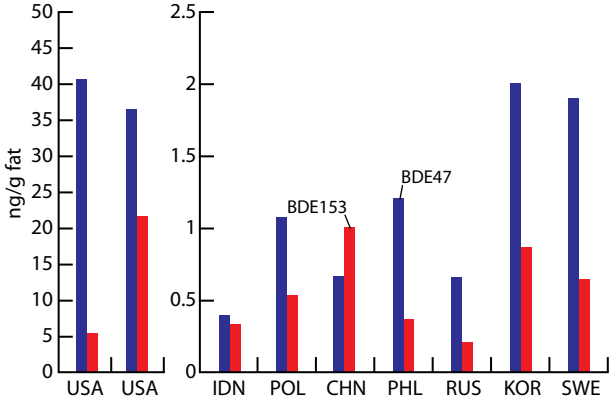


Figure 3.38. Examples of concentrations (ng/g fat) reported for two PBDE congeners, BDE-47and BDE-153, in mothers' milk from eight different countries (Country codes according to ISO 3166/MA Alpha-3-code). The two USA studies represent concentrations of the two PBDE congeners in milk from mothers in Massachusetts, USA (left USA bar) (Johnson-Restrepo et al., 2007) and from mothers in the USA in general (USA right bar) (Schecter et al., 2010).

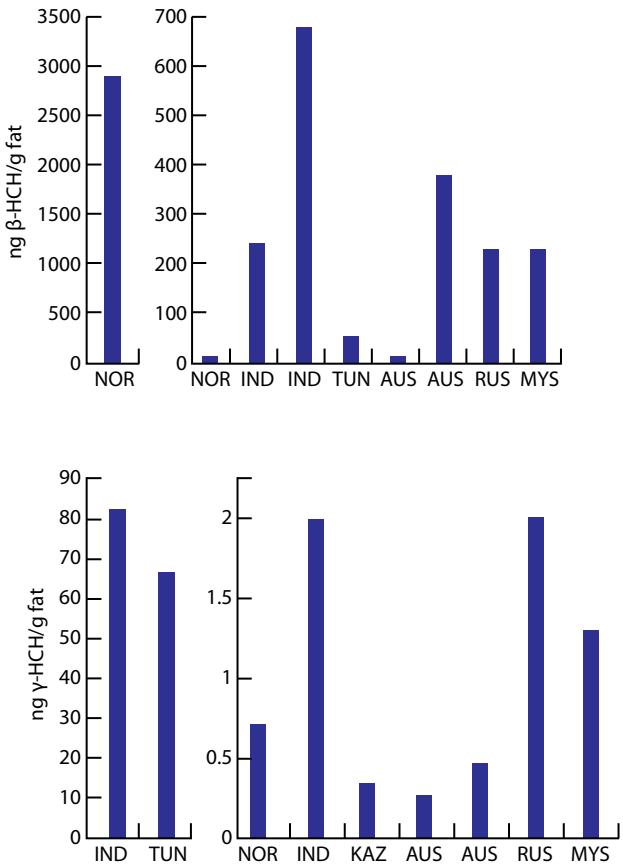


Figure 3.39. Concentrations (ng/g fat) of β-HCH (upper diagram) and γ-HCH (Lindane) (lower diagram) in mothers' milk from selected countries worldwide (Country codes according to ISO 3166/MA Alpha-3-code). Data are from WHO mothers'milk programme (UNEP, 2009c; 2011b).

this compound appear to be prevalent in milk. Conjugation (e.g. glucuronidation, sulfation) is a defense mechanism able to reduce the potential toxicity of compounds when only the free species is bioactive, as it is the case for bisphenol A. Accordingly, only free bisphenol A is usually measured in monitoring programmes used for human exposure assessment. However, most of the ingested conjugates are hydrolyzed during the digestive process, including in infants, although the phenomenon occurs to a limited extent in babies as compared with adults (Franke et al., 2006). The hydrolysis can take place in the stomach, due to the action of hydrochloric acid, but requires mainly the action of gut microflora to cleave conjugated bisphenol A (and other conjugates) to the free bisphenol A, prior to its intestinal absorption. For these reasons, exposure of breast fed infants should be based on total bisphenol A concentrations in milk and not only on free species.

Cariou et al. (2008) analyzed tetrabromobisphenol A (TBBPA) in mothers' milk samples collected in France and found levels varying from 0.062 to 37.000 ng/g fat (median value = 0.5 ng/g fat). These values are approximately one or two orders of magnitude higher than concentrations observed by other authors in various countries (Abdallah & Havrad, 2011;

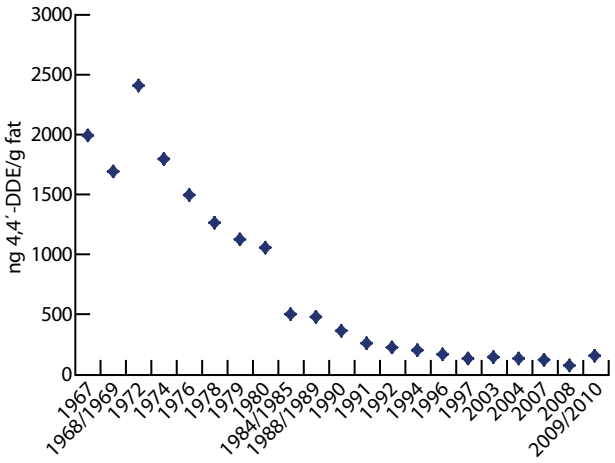


Figure 3.40. Temporal trends of 4,4'-DDE concentrations (ng/g fat) in Swedish mothers' milk (Norén & Meironyté, 2000; Bergman et al., 2010).

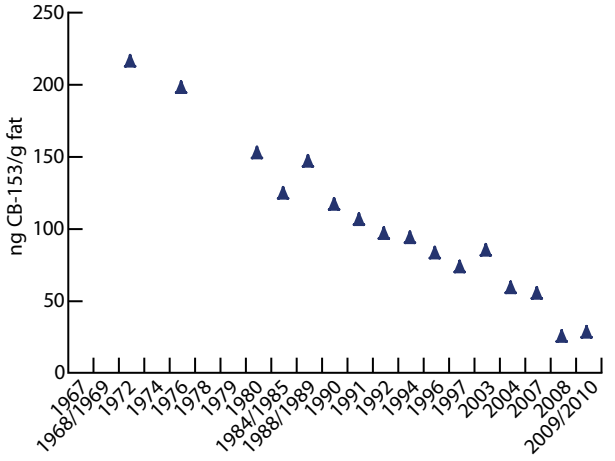


Figure 3.41. Temporal trends of CB-153 concentrations (ng/g fat) in Swedish mothers' milk 1972 - 2010 (Norén & Meironyté, 2000; Bergman et al., 2010).

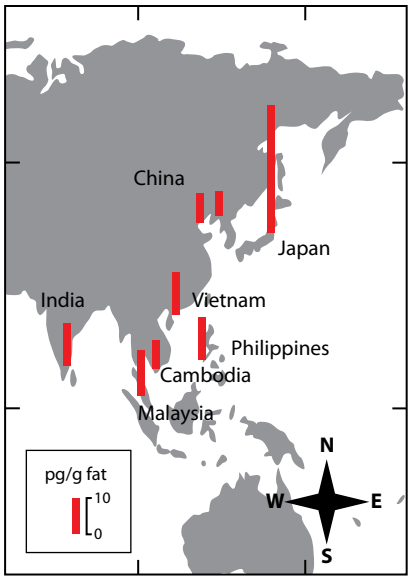


Figure 3.42. PCDD/PCDF and dioxin-like PCBs TEQ concentrations (pg/g fat) in mothers' milk from seven Asian countries (Zheng et al., 2008a) (Graph redrawn; Used with publisher's permission).

Shi et al., 2009; Thomsen, Lundanes & Becher, 2002). The apparent discrepancy between these studies is probably due to the fact that Cariou and co-workers included a hydrolysis step in the sample preparation procedure that was not done by other groups. This also suggests, as previously described for bisphenol A, that a major fraction of TBBPA found in mothers' milk is most likely present as TBBPA conjugates.

3.2.3 Conclusions

EDCs in wildlife and humans

- Exposure assessments of POPs and mercury in wildlife cover more of the globe today than a decade ago. This indicates increased monitoring of EDC exposures in wildlife. However, there are still major data gaps for POPs and mercury, particularly in tropical and subtropical areas.
- Newer information shows that wildlife are being exposed to a much greater diversity of chemicals in the environment than was documented ten years ago. In particular, pharmaceutical and personal care product ingredients and halogenated phenolic compounds are now commonly reported in wildlife.
- High levels of several POPs are still found in top predators in polar regions due to long-range transport and deposition of these chemicals and food web biomagnification.
- Several brominated flame retardants and perfluorinated surfactants have also become focal issues over this decade and are found globally in many different wildlife species. Unlike other POPs, PBDEs and PFOS are generally highest in wildlife near urban areas around the globe.
- Mussels have been widely used for POPs and metals monitoring and show promise for determining spatial and temporal trends of EDCs that are less persistent like bisphenol A, alkyl phenols and PAHs.
- Monitoring of abiotic media such as surface waters, soils, dust, and sediments near sources is also needed for assessing wildlife and human exposure to less persistent EDCs.
- The primary biological matrices in humans for measuring POPs are blood and mothers' milk, for POPs metabolites is blood, and for less persistent and less bioaccumulative chemicals is urine, due to the short half-lives in humans.
- Concentrations of EDCs in humans are strongly affected by activities, diet, nutritional status, and the places where people live, work and play. Concentrations of some EDCs (e.g. PBDEs) are higher in young children because of their high hand-to-mouth activities.
- Human and wildlife exposure to EDCs prior to and during pregnancy (prenatal exposure) is of particular concern due to the vulnerability of the developing fetus. Pregnant females are exposed to multiple chemicals. Most chemicals can cross the placenta, leading to fetal exposure.

- Wildlife and human infants can be exposed to EDCs via mothers' milk.

Changes in EDCs over time

A decade ago, the majority of data on human internal exposure to EDCs was on legacy POPs such as DDTs, PCBs and HCB. Since then, numerous reports on other POPs (e.g. PBDEs, PFOS), and chemicals used in materials and consumer products (e.g. phthalates, triclosan, siloxanes, bisphenol A, parabens) in human samples have been published for some countries. It is clear that humans are being exposed to a diverse mixture of EDCs.

- Global data on human concentrations of some POPs (e.g. chlorinated paraffins, mirex, toxaphene and endosulfan) and the less persistent, less bioaccumulative chemicals (e.g. phthalates, bisphenol A) are lacking.
- Long-term temporal trends of EDCs in wildlife and humans are available only for some POPs and mercury, and only in very few areas of the world.
- Over time, several POPs (e.g. PCBs, PBDEs and PFOS) have increased and then more recently decreased in most areas where concentrations in wildlife were measured. These decreases are due to restrictions or bans on their use in many countries.
- Temporal trend data for POPs are available only from a few human populations in the world. The data suggest that levels of the POPs that have been banned, or restricted in their use, are declining.
- There are very limited exposure and temporal trend data for less persistent, non-bioaccumulative EDCs in wildlife and humans.

3.3 Emerging issues and EDCs of concern

3.3.1 Identification of EDCs from chemicals in commerce

Identifying endocrine active chemicals from among the chemicals in commerce worldwide is a major challenge (Phillips et al., 2008). The EDCs identified so far (e.g. in TEDX, 2011) have a wide diversity of molecular structures. Selection of chemicals for detailed screening has been based on expert judgment using available toxicology data. However, as the USA EPA Endocrine Disrupting Screening Program (EDSP) website has noted, with the exception of food-use and consumer pesticides with regulatory mandates requiring prenatal developmental and two-generation reproductive toxicity testing, substantial endocrine effects data are lacking for most chemicals (US EPA, 2011c). Selection of potential EDCs from European chemical lists has also used a combination of exposure modeling and expert judgment to identify about 550 substances (Petersen, Rasmussen & Gustavsen, 2007).

There are over 143,000 chemicals in commerce based on the preregistrations done as part of the REACH legislation in the European Union (ECHA, 2012), although early estimates suggested that only about 30,000 produced or imported in quantities of one metric ton would require full registration (European Commission, 2003). In the USA there are 84,000 registered chemicals (including polymers) under the Toxic Substances Control Act (TSCA) inventory (>4.5 t/yr), although not all of these chemicals may be currently in production (USA EPA, 2011b). The identities of 17,000 inventory listings do not appear on the public version of the TSCA inventory because manufacturers have claimed the chemicals' identities as confidential business information (Denison, 2007). The Chinese "Inventory of Existing Chemical Substances in China" includes about 45,000 substances (http://www.crc-mep.org.cn/iecscweb/IECSC.aspx?La=1); however, this is thought to be only 30-50% of the chemicals likely imported or produced in China (http://www.rsc.org/chemistryworld/News/2010/April/01041001.asp). India has an inventory of hazardous chemicals (Government of India, 2008); however, no firm number of chemicals in commerce is yet available from this country. The large and growing production of chemicals in China and India, and their incorporation into consumer and industrial materials and goods for global export add to the challenge of defining the full numbers of chemicals in commerce.

Based on present knowledge, it is possible to trace high production volume chemicals to their application areas, but that is not the case for numerous additives and process chemicals. Adding greatly to the complexity are the unknown or unintended by-products that are formed during chemicals manufacturing, during combustion processes, and via environmental transformations, thus adding to the number of chemicals present in the environment. While the active ingredients in pharmaceuticals and pesticides have to be documented on the final product, this is not the case in materials and goods for construction, down-stream manufacturing and consumption.

The physical-chemical characteristics and reactivity of the individual chemicals in commerce range from low molecular masses that are highly water soluble and volatile to molecular masses up to around 1000 Daltons that are poorly water soluble, non-volatile, and either neutral or ionizable. Their reactivity goes from highly reactive to almost inert. All organic chemicals have a certain half-life in each of the environmental compartments and in vivo, due to metabolism. Further, almost all chemicals are transformed abiotically and biologically to numerous other chemicals (transformation products (TPs)). Accordingly, the chemosphere consists of compounds with the characteristics listed in **Table 3.1**, being persistent and bioaccumulative and undergoing long-range transport, or being stable enough to expose humans and wildlife to them even though their half-lives are short. Finally, there are the most reactive chemicals with very short half-lives in the

abiotic environment and in biota. These compounds will form more stable TPs than their parent compounds and adducts to biomacromolecules.

The range of properties of known EDCs based on the TEDX list (TEDX, 2011) is illustrated in **Figure 3.41** and compared against their environmental bioaccumulation potential to humans (EBAP) (Czub & McLachlan, 2004). EBAP is the ratio of the quantity of a chemical in a human to the quantity of the chemical present in 1 m^2 of the environment. Highly bioaccumulative chemicals have a maximum EBAP at log K_{OW} ~7 and log K_{OA} ~9. The majority of known EDCs (555 of 792 organic chemicals with known structures) are found within the chemical space of >10% maximum EBAP (log K_{OW} >3 and <10; log K_{OA} >6), indicating that they can accumulate in humans and in the agricultural and marine food webs. However, a significant fraction of the TEDX list (30%) consists of chemicals with predicted properties that are outside of this range (**Figure 3.43**).

Thus, selecting all chemicals with some potential to accumulate in human and wildlife food webs, along with knowledge of toxicology, is one approach to identify chemicals for further assessment. The screening of hundreds of thousands of chemicals in commerce has been done largely with a focus on persistent and bioaccumulative chemicals (Howard & Muir, 2010; Brown & Wania, 2008). As quantitative structure-activity relationships (QSARs) are developed for various endocrine endpoints, it will be possible to screen these lists again and again for various structures with known biological activity. However, with limited or no toxicological data on the vast majority of the chemicals, the development and validation of suitable QSARs is a challenge (Cronin and Worth, 2008). For example for the TEDX list of 850 chemicals with known effects on the endocrine system, 30% are halogenated phenolic compounds, indicating the importance of testing chemicals with these structures for estrogenic and thyroidogenic effects. These structures can be readily identified (Walker et al., 2003) and QSARs models have been developed for several endocrine receptor-related in vitro endpoints (reviewed by Schmieder et al., 2003). A greater challenge is the identification of novel EDCs and novel modes of action given the limited domains of existing QSAR training sets (Phillips et al., 2008); some chemicals may not be identified as EDCs using the current QSAR approaches given the diverse modes of actions that these chemicals can have on the endocrine system (Chapter 1).

The most obvious approach to initiate measurements of potential novel EDCs is a better global declaration of chemicals in materials and goods. Since the knowledge is, or should be, in place on the manufacturing side, it is reasonable to not only apply that to, e.g. pharmaceuticals, pesticides and cosmetics, but also to the chemicals going into construction materials, textiles, electronics and other consumer materials and goods. As such, structure-based selections of chemicals and preparation of highly pure standards for their analysis and testing can be done. Until proper documentation is available on all products, the scientific and regulatory communities need to work both ways, using the information available on chemicals in products and the well-designed and directed chemical analytical and bio analytical pathways.

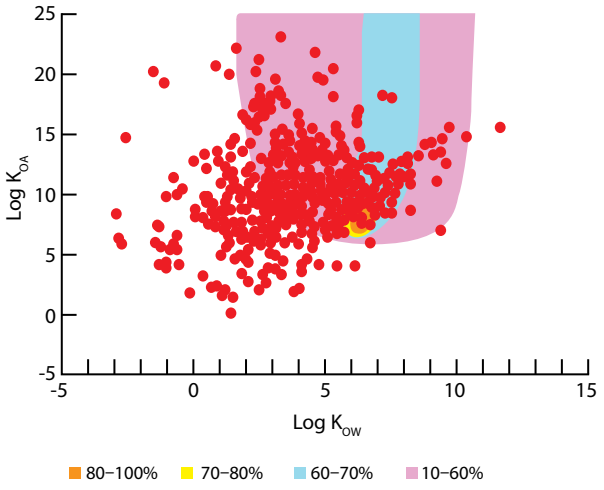


Figure 3.43. Log K_{OW} and log K_{OA} values for 792 EDCs from the TEDX (2011) EDC list are overlaid on the environmental bioaccumulation potential (EBAP; coloured areas) of persistent chemicals for a marine and agricultural diet (Czub & McLachlan, 2004). The coloured regions represent the percentage of the maximum EBAP obtained within the chemical partitioning space. The log K_{OA} values were estimated using EPISuite 4.1 KOAWIN software (US EPA, 2011a). The K_{OW} values were based on measured values for 421 EDCs and values predicted using EPISuite KOWIN for the others.

3.3.2 Analytical challenges in identifying, quantifying and reporting EDCs

Wildlife and humans are exposed to a wide variety of EDCs that differ greatly in their physical-chemical properties. Further, these compounds are generally present at very low levels and in complex matrices requiring highly selective and sensitive methods. It is clear that very low exposures to chemicals can have an effect on the endocrine system (see Chapter 1) and it is critical to be able to quantify these exposures. The wide range of different compound classes requires a variety of analytical approaches and techniques. In general, complicated and time-consuming extraction and purification steps are required, followed by chromatographic techniques often coupled to mass spectrometry. The majority of established analytical methods focus on the specific classes listed in **Table 3.1** and closely related compounds, e.g. dioxins and dioxin-like PCBs, indicator PCBs, organochlorine pesticides, BFRs, PFCs, phthalates, bisphenol A, and a large number of halogenated phenolic and non-phenolic compound classes known to exert ED effects (TEDX, 2011). Indeed, large numbers of halogenated phenols have been identified both in wildlife and in humans (Athanasiadou et al., 2008; Letcher, Klasson-Wehler & Bergman, 2000; Hovander et al., 2002). For routine quantitative analysis, however, analytical standards are required and, while they are available for most pesticides,

pharmaceuticals, PAHs, metals and many halogenated organics with POPs characteristics, they are typically not available for TPs nor for the vast array of organic chemicals in commerce (Howard & Muir, 2010). Thus targeted analyses based on analytical standards provide the necessary sensitivity and selectivity, but lead to a fragmented picture of the occurrence of EDCs in the environment, wildlife and humans. Consequently, there is a high demand for developing screening analytical methods that will accommodate a wide variety of analytical functional groups at low detection levels.

Successful chemical analyses are built on methods development, and should not rely only on instruments with lower and lower detection limits. Since POPs have been target analytes for a long time it is here the most advanced techniques for detection and quantification are available. Better methodologies are required for chemicals with variable persistence and short half-lives in vivo. These are adding up to a very large number of chemicals with highly different structures. These are the chemicals for which new methods need to be developed and the analytes need to be characterized in detail to promote methodological advances. Hence pure compounds are required. HBCDD may serve as an example of how analysis of a simple molecule has become increasingly complex with time (EFSA, 2011b; Law et al., 2005). Without detailed chemical characterization, it would not have been possible to improve the quality of the analyses and exposure assessments. While commercial chemicals can be obtained and purified, there is a major demand for chemical synthesis of metabolites and abiotically transformed products.

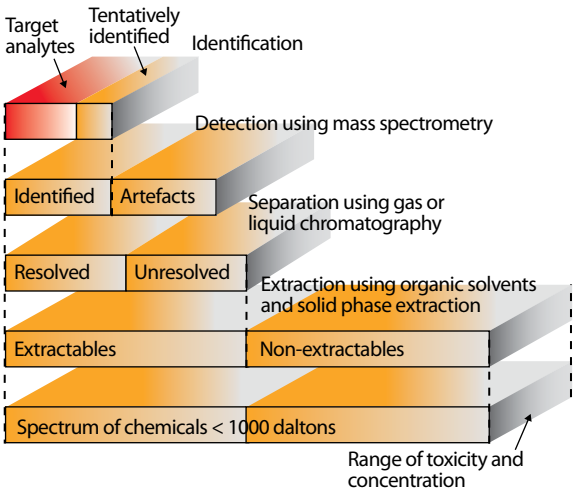


Figure 3.44. An illustration of the complexity of measuring chemicals, including potential EDCs, in environmental media. While the "spectrum of chemicals" (not isomers or congeners) is very large, only a subset can be extracted and separated by chromatography, and even fewer identified. [redrawn from Daughton, 2005; GC – gas chromatography, LC- liquid chromatography, MS – mass spectrometry, SPE – solid phase extraction]

The analysis of target analytes can be viewed as a top-down approach that only scratches the surface of the number of chemicals that can be measured as illustrated by **Figure 3.44**. Larger numbers can be tentatively identified based on mass spectra, but authentic standards are ultimately needed. Even larger numbers are isolated by the conventional extraction and separation technology widely employed in trace organics analysis laboratories, but they cannot be readily identified. Furthermore, the analyst has to make a decision about how to best allocate analytical resources for this task. The use of effects directed analysis (EDA) and QSAR directed non-target analyses are two emerging techniques that are helping to address this challenge (Hecker & Hollert, 2009; Helbling et al., 2010; Schymanski et al., 2009).

EDA involves bioassay-directed fractionation techniques to decrease the complexity of the sample matrix before identification of the endocrine active compounds. Estrogenic and androgenic in vitro assays are typically employed to screen fractions of sediment or wastewater extracts that were previously separated by liquid chromatography (Hecker & Hollert, 2009; Weiss et al., 2009; 2011). QSAR directed analysis involves identification of transformation products from structural information and identification from full scan high resolution mass spectra using post-acquisition data processing. Non-target analyses of candidate chemicals for which no standards are available can sometimes be accomplished by very high resolution mass spectrometry. However improved prediction systems for theoretical fragmentation patterns, retention times, and ionization behaviour are needed to widely apply this technology (Krauss, Singer & Hollender, 2010).

Even though wildlife and human matrices are available for extraction and analysis, the question remains on how measured levels are to be translated into an internal dose. Since chemical analyses and exposure assessments still lack standardization, it is only possible to do limited comparisons between studies. This lack of standardization is also hampering assessments of effects of mixtures. In this context, it is necessary to point out that it is not the mass-based concentrations that count when mixture doses are assessed, but the number of molecules. Hence, levels of contaminants in humans and wildlife need to be compared on a molar basis.

Human biomonitoring is a valuable tool in exposure assessment but it is usually performed at a single time point that disregards the variation in exposures throughout life and critical time periods. Recently, the concept of "exposome" has been introduced in the investigation of human exposure to environmental contaminants, representing the total exposure from conception onwards being of critical interest for understanding the environmental causes for disease (Rappaport, 2011; Wild, 2005). The comprehensive measurements of all exposure events during a lifetime requires ongoing, longitudinal sampling, particularly during critical life stages such as fetal development, early childhood and the reproductive years.

3.3.3 Conclusions

- While large lists of chemicals in commerce are now available and can be searched electronically, it is difficult to identify potential EDCs from among these chemicals because there is a lack of endocrine effects data on which to build suitable QSARs.
- Even for known or potential EDCs there is still a lack of data on where these chemicals are produced and used in products, materials and goods. This limits our ability to identify where and how much EDC might be in the environment or in wildlife and humans.
- Only a few of all potential EDCs are measured in the environment and in people and wildlife. Further, there is little known about metabolites of EDCs and how EDCs are transformed in products, which limits our ability to identify and measure them in wildlife, humans and the environment.
- The majority of identified EDCs have properties that suggest they will accumulate in humans and in agricultural/ aquatic food webs. However these EDCs may not be representative of the full range of molecular structures and properties of potential and known EDCs, due to the previous narrow focus on testing only halogenated chemicals for their estrogenic and thyroidogenic effects.
- The lack of appropriate methods for measuring many industrial chemicals, pesticides, pharmaceuticals, etc., is a major obstacle for exposure assessments of potential EDCs.
- The use of effects directed analysis as well as non-target analysis with high resolution mass spectrometry are emerging techniques that are helping to address the issue of lack of knowledge of specific chemicals or transformation products.
- Since there are no standards for how concentrations are to be reported for either wildlife or human matrices, this is strongly hampering comparisons between studies and further exposure assessments on a general basis.

3.4 Main messages

1. **EDCs are everywhere** - EDCs are chemically diverse, primarily include human-made chemicals, and are used in a wide range of materials and goods. EDCs are present in food, nature and human beings. They can also be formed in the environment and in humans, wildlife and plants.
2. **Increasing number of EDCs** – Unlike ten years ago, it is better understood that humans and wildlife are exposed to far more EDCs than just persistent organic pollutants (POPs). However, only a fraction of the potential EDCs in the environment are currently understood.
3. **Exposed to mixtures** - Humans and wildlife are also exposed to multiple EDCs at the same time, and there is justifiable concern that different EDCs can act together and result in an increased risk of adverse effects on human health and wildlife.
4. **Still measuring only a few** - Right now only a narrow spectrum of chemicals and a few classes of EDCs are measured, making up the tip of the iceberg. More comprehensive assessments of human and wildlife exposures to diverse mixtures of EDCs are needed. It should be a global priority to develop the abilities to measure any potential EDCs. Ideally, an "exposome" should be developed, i.e. a highly detailed map of environmental exposures that might occur throughout a lifetime.
5. **Exposure occurs at early life stages** - Exposures to EDCs occur during vulnerable periods of human and wildlife development – from fertilization through fetal development and through nursing of young offspring - and raises particular concern.
6. **Important routes of exposure -** New routes of exposure to EDCs, in addition to food intake, have been identified and include indoor environments and electronics recycling and dumpsites in developing countries. Children can have higher exposures due to their hand-to-mouth activities. For some EDCs, all of the routes of exposure are not understood.
7. **All sources of EDCs not known** – All sources of exposure to EDCs are not understood because of the lack of chemical constituent declarations for materials and goods.
8. **Importance of biotic and abiotic environmental monitoring** – Spatial and temporal monitoring is critical for understanding trends and levels of exposure. This monitoring should include both tissues from humans and wildlife (representing a range of species), as well as water or other environmental compartments to capture the less persistent EDCs.
9. **Changes in use lead to changes in levels** – Levels in humans and wildlife are related to how much a chemical is used. Bans on several POPs have led to declines in environmental levels and human body burdens. In contrast, there are increasing levels of some newer EDCs such as perfluorinated alkyl compounds and replacements for banned brominated flame retardants.
10. **Global movement of EDCs** - There is global transport of EDCs through natural processes (ocean and air currents) as well as through commerce, leading to worldwide exposure of humans and wildlife to EDCs**.**

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Appendix I. Common names and Latin names of species mentioned in the present document.

African clawed frog	*Xenopus laevis*
African darter	*Anhinga rufa*
American alligator	*Alligator mississippiensis*
American kestrel	*Falco sparverius*
American robin	*Turdus migratorius*
American toad	*Bufo americanus*
American white ibis	*Eudocimus albus*
Antartic fur seal	*Arctocephalus gazella*
Arctic char	*Salvelinus alpines*
Arctic fox	*Vulpes lagopus*
Arctic glaucous gull	*Larus hyperboreus*
Atlantic croaker	*Micropogonias undulates*
Atlantic white–sided dolphin	*Lagenorhynchus acutus*
Atlantic white–sided dolphin	*Lagenorhynchus acutus*
Atlantic(bottlenose) dolphin	*Tursiops truncatus*
Bacteria	*Aeromonas hydrophila*
Bacteria	*Aeromonas salmonicida*
Baikal seal	*Pusa sibirica*
Bald Eagle	*Haliaeetus leucocephalus*
Bearded seal	*Erignathus barbatus*
Beluga or White whale	*Delphinapterus leucas*
Beluga whales	*Delphinapterus leucas*
Black deer (Sitka black tail deer)	*Odocoileus hemionus sitkensis*
Black-crowned night heron	*Nycticorax nycticorax*
Blackfooted albatross	*Phoebastria nigripes*
Blue mussel	*Mytilus edulis*
Bluegill	*Lepomis macrochirus*
Brown bullhead	*Ameiurus nebulosus*
Brown shrimp	*Crangon crangon*
Bull frog	*Rana catesbeiana*
Burmeister's porpoise	*Phocoena spinipinnis*
Buzzard	*Buteo buteo*
Californian sea lion	*Zalophus californianus*
Caribou	*Rangifer tarandus*
Carp (common)	*Cyprinus carpio*
Cat	*Felis catus*
Cattle	*Bos primigenius*
Chicken	*Gallus domesticus*
Chinook salmon	*Oncorhynchus tshawytcha*
Clam (soft-shelled)	*Mya arenaria*
Clapper rail	*Rallus longirostris*
Colonial ascidian	*Botryllus schlosseri*
Common bottlenose dolphin	*Tursiops truncatus*
Crow	*Corvus brachyrhynchos*
Deer mice	*Peromyscus maniculatus*
Dog	*Canis lupus*
Double crested cormorant	*Phalacrocorax auritis*
Dusky dolphin	*Lagenorhynchus obscurus*
Earthworm	*Lumbricus terrestris*
Eastern bluebird	*Sialia sialis*
Eastern oyster	*Crassostrea virginica*
Echinoid	*Paracentrotus lividus*
Eland	*Tragelaphus oryx*
Eland(common)	*Taurotragus oryx*
Elephant	*Loxodonta africana/ Elephas maximus*
Estuarine bivalve	*Scrobicularia plana*
European harbor porpoise	*Phocoena phocoena*
European shag	*Phalacrocorax aristotelis*
False killer whale	*Pseudorca crassidens*
Fathead minnow	*Pimephales promelas*
Finless porpoise	*Neophocaena phocaenoides*
Flounder	*Paralichthys olivaceus*
Franciscana dolphin	*Pontoporia blainvillei*
Fraser's dolphin	*Lagenodelphis hosei*
Freshwater amphipod	*Gammarus pulex*
Freshwater hydroid	*Hydra vulgaris*
Freshwater water flea	*Daphnia magna*
Fulmar	*Fulmarus glacialis*
Galapagos sea lion	*Zalophus wollebaeki*
Ganges river dolphin	*Platanista gangetica gangetica*
Gizzard shad(American)	*Dorosoma cepedianum*
Goat (domestic)	*Capra aegagrus hircus*
Goldfish	*Carassius auratus auratus*
Great blue heron	*Ardea herodias*
Great cormorant	*Phalacrocorax carbo*
Great egret	*Ardea alba*
Grey seal	*Halichoerus grypus*
Guillemot	*Uria aalge*
Guinea pig	*Cavia porcellus*
Harbour seal	*Phoca vitulina*
Herring	*Clupea harengus*
Herring Gull (American)	*Larus smithsonianus*
Herring gull(European)	*Larus argentatus*
Horse	*Equus caballus*
Housefly	*Musca domestica*
Human	*Homo sapiens*
Humpback dolphin (Chinese)	*Sousa chinensis*
Humpback dolphin (Indian)	*Sousa plumbea*
Humpback dolphin(Atlantic)	*Sousa teuszi*
Indo-pacific dolphin	*Tursiops aduncus*
Indo-pacific humpback dolphin	*Sousa chinensis*
Isopod	*Porcellio scaber*

Ivory gull	*Pagophila eburnea*
Jaguar	*Panthera onca*
Japanese medaka	*Oryzias latipes*
Killer whale	*Orcinus orca*
Lesser black-backed gull	*Larus fuscus fuscus*
Lion	*Panthera leo*
Lobster	*Homarus americanus*
Long beaked common dolphin	*Delphinus capensis*
Loon (common)	Gavia immer
Mallard	*Anas platyrhynchos*
Mediterranean mussel	*Mytilus galloprovincialis*
Melon headed whale	*Peponocephala electra*
Mink	*Mustela vison*
Minke whale	*Balaenoptera acutorostrata*
Mosquitofish	*Gambusia affinis holbrooki*
Mouse	*Mus musculus*
Mudpuppy (common)	*Necturus maculosus*
Mummichog	*Fundulus heteroclitus*
Mussel	*Elliptio complanata*
Neogastropod	*Thais clavigera*
Northern fur seal	*Callorhinus ursinus*
Northern pike	*Esox lucius*
Osprey	*Pandion haliaetus*
Otter	*Lutra lutra*
Owl limpet (sea snail)	*Lottia gigantean*
Pacific white-sided dolphin	*Lagenorhynchus obliquider*
Perch	*Perca fluviatilis*
Peregrine falcon	*Falco peregrinus peregrinus*
Pinnipeds	Seals, sea lions and walrus
Polar bear	*Ursus maritimus*
Polar seastar	*Leptasterias polaris*
Pumpkinseed sunfish	*Lepomis gibbosus*
Rabbit	*Oryctolagus cuniculus*
Rainbow trout	*Oncorhynchus mykiss*
Ramshorm snails	*Marisa cornuarietis*
Rat	*Rattus norvegicus*
Ringed seal	*Pusa hispida*
Risso's dolphin	*Grampus griseus*
River otter	*Lutra canadensis*
Roach	*Rutilus rutilus*
Rock shell	*Thais clavigera*
Rough woodlouse	*Porcellio scaber*
Sand dollar	*Echinodermata: Echinoidea*
Sand goby	*Pomatoschistus minutus*
Sea otter	*Enhydra lutris*
Sea slug	*Aplysia*
Sea star	*Asterias rubens*
Sea turtle (green)	*Chelonia mydas*
Sea urchin	*Phylum echinodermata*
Sharptooth catfish	*Clarias gariepinus*
Sheep	*Ovis aries*
Short-beaked common dolphin	*Delphinus delphi*
Skipjack tuna	*Katsuwonus pelamis*
Southern catfish	*Silurus meridionalis*
Spotted seal	*Phoca largha*
Star ascidian	*Botryllus schlosseri*
Steller sea lion	*Eumetopias jubatus*
Striped dolphin	*Stenella coeruleoalba*
Subantartic fur seal	*Arctocephalus tropicalis*
Tasmanian devil	*Sarcophilus harrisii*
Thickbilled murre (Brünnich's Guillemot)	*Uria lomvia*
Tiger	*Panthera tigris*
Tree sparrow	*Passer montanus*
Tree swallow	*Tachycineta bicolour*
Tucuxi dolphin	*Sotalia fluviatilis*
Tunicate	*Styela plicata*
Vase tunicate	*Ciona intestinalis*
Viviparous blenny	*Zoarces viviparus*
Walrus	*Odobenus rosmarus*
Water flea	*Daphnia magna* or *Daphnia pulex (another species)*
White stork	*Ciconia ciconia*
White sucker	*Catostomus commersoni*
White tailed deer	*Odocoileus virginianus*
White-beaked dolphin	*Lagenorhynchus albirostris*
Yellow perch	*Perca flavescens*
Zebra mussel	*Dreissena polymorpha*
Zebrafish	*Danio rerio*

Appendix II. The table includes common names, abbreviations, when applicable, chemical names of those chemicals that are mentioned in the text of the Chapters 1-3. Chemical Abstract System numbers (CAS #) are given for further information on each of the chemicals as well as class of compound and/or major use.

Common names	Chemical name; or other common name	CAS #	Class or use	Abbreviation
Acetochlor	2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide	34256-82-1	Herbicide	
Alachlor	2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	15972-60-8	Herbicide	
Amitrole	1,2,4-Triazol-3-amine aminotriazole	61-82-5	Herbicide	
Anthracene	Paranaphthalene	120-12-7	PAH	
Aroclor 1254	Polychlorinated biphenyls	11097-69-1	PCB mixture	
Arsenic (As)	-	7440-38-2	Heavy metal	
Atrazine	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	1912-24-9	Herbicide	ATR
Benzo(a)anthracene	Benzanthracene; Benzanthrene; 1,2-Benzanthracene; Benzo[b] phenanthrene; Tetraphene	56-55-3	PAH	BaA
Benzo(a)pyrene	3,4-Benz[a]pyrene	50-32-8	PAH	BaP
BB-153	2,2′,4,4′,5,5′-Hexabromobiphenyl	59080-40-9	PBB	
Benzyl butyl phthalate	n-Benzyl butyl phthalate	85-68-7	Phthalate	BBP
BDE-209	Decabromodiphenyl ether	1163-19-5	PBDE	decaBDE
BDE-47	2,2′,4,4′-Tetrabromodiphenyl ether	5436-43-1	PBDE	
BDE-99	2,2′,4,4′,5-Pentabromodiphenyl ether	60348-60-9	PBDE	
Benzene	1,3,5-Cyclohexatriene	71-43-2	Aromatic solvent	
Benzylidene camphor	(3*E*)-1,7,7-Trimethyl-3-[(4-methylphenyl methylene]-2-norbornanone	36275-29-3	UV filter	
Bisphenol A	2,2-Bis(4-hydroxyphenyl)propane	80-05-7	Plastics monomer	BPA
Bisphenol A diglycid ether	2-[[4-[2-[4-(Oxiran-2-ylmethoxy)phenyl]propan-2-yl]phenoxy] methyl]oxirane	1675-54-3	Plastics monomer	
Bisphenol F	Bis(4-hydroxydiphenyl)methane	87139-40-0	Plastics monomer	BPF
Bisphenol S	4,4′-Sulfonylbisphenol	80-09-1	Plastics monomer	BPS
Bromacil	5-Bromo-3-(butan-2-yl)-6-methylpyrimidine-2,4(1H,3H)-dione	314-40-9	Herbicide	
Butylate	S-Ethyl diisobutyl(thiocarbamate)	2008-41-5	Herbicide	
Butylated hydroxyanisole	2(3)-tert-Butyl-4-hydroxyanisole	25013-16-5	Antioxidant	BHA
Cadmium (Cd)	Cadmium chloride	10108-64-2	Heavy metal	
Carbamazepine	5H-Dibenzo[b,f]azepine-5-carboxamide	298-46-4	Pharmaceutical	
Carbaryl	1-Naphthyl methylcarbamate	63-25-2	Insecticide	
CB-15	4,4′-Dichlorobiphenyl	2050-68-2		
CB-77	3,3′,4,4′-Tetrachlorobiphenyl	32598-13-3	Planar PCB	
CB-118	2,3′,4,4′,5-Pentachlorobiphenyl	31508-00-6		
CB-126	3,3′,4,4′,5-Pentachlorobiphenyl	57465-28-8	Planar PCB	
CB-132	2,2′,3,3′,4,6′-Hexachlorobiphenyl	38380-05-1		
CB-138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	35065-28-2		
CB-153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	35065-27-1		
CB-169	3,3′,4,4′,5,5′-Hexachlorobiphenyl	32774-16-6	Planar PCB	
CB-180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	35065-29-3		
Chlordane	cis/trans-Chlordane	5103-71-9, 5103-74-2	Organochlorine Insecticide	
Chlordibromomethane	Chlordibromomethane	124-48-1	Trihalomethane	
Chlorinated Paraffins	Polychlorinated alkanes		Flame retardants, Lubricants, Plasticizers	CPs or PCAs
Chlorpyrifos	O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate	2921-88-2	Insecticide	
Citalopram	(RS)-1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile	59729-33-8	Pharmaceutical	
Clofentezine	3,6-Bis(2-chlorophenyl)-1,2,4,5-tetrazine	74115-24-5	Pesticide/Acaracide	
Coumaphos	O,O-Diethyl O-3-chloro-4-methyl-2-oxo-2H-chromen-7-yl phosphorothioate	56-72-4	Pharmaceutical	

Coumestrol	3,9-Dihydroxy-6-benzofurano[3,2-c]chromenone	479-13-0	Coumestans/ Phytestrogen	
D4	Octamethylcyclotetrasiloxane	556-67-2	Cyclic siloxane	
D5	Decamethylcyclopentasiloxane	541-02-6	Cyclic siloxane	
D6	Dodecamethylcyclohexasiloxane	540-97-6	Cyclic siloxane	
Daidzein	7-Hydroxy-3-(4-hydroxyphenyl) chromen-4-one	486-66-8	Isoflavones/ phytestrogen	
Dibromochloropropane	1,2-Dibromo-3-chloropropane	96-12-8	Pesticide/Soil Fumigant	DBCP
Desethylatrazine	4-Amino-2-chloro-6-isopropylamino-s-triazine	6190-65-4	Herbicide Metabolite	DEA
2,4-D	2,4-Dichlorophenoxy)acetic acid	94-75-7	Herbicide	2,4-D
2,4-Dichlorophenol	1,3-Dichloro-4-hydroxybenzene	120-83-2	Chlorophenol	2,4-DCP
3-Diltiazem	cis-(+)-[2-(2-Dimethylaminoethyl)-5-(4-methoxyphenyl)-3-oxo-6-thia-2-azabicyclo[5.4.0]undeca-7,9,11-trien-4-yl] ethanoate	42399-41-7	Pharmaceutical	
2,4′-DDD (o,p′-DDD)	2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane	53-19-0	Organochlorine Insecticide	o,p′-DDD 2,4′-DDD
2,4′-DDT (o,p′-DDT)	2-(2-Chlorophenyl)-2-(4-chlorophenyl-1,1,1-dichloroethane	789-02-6	Organochlorine Insecticide	o,p′-DDT 2,4′-DDT
4,4′-DDD (p,p′-DDD)	2,2-Bis-(4-chlorophenyl)--1,1-dichloroethane	72-54-8	Organochlorine Insecticide	p,p′-DDD 4,4′-DDD
4,4′-DDE (p,p′-DDE)	2,2-Bis-(4-chlorophenyl)-1,1-dichloroethene	72-55-9	Organochlorine Insecticide	p,p′-DDE 4,4′-DDE
4,4′-DDT (p,p′-DDT)	2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethane	50-29-3	Organochlorine Insecticide	p,p′-DDT 4,4′-DDT
Di-(2-ethylhexyl)adipate	Bis(2-ethylhexyl)adipate	103-23-1	Plasticizer	DEHA
Dehydroepiandrosterone	(3S,8R,9S,10R,13S,14S)-3-Hydroxy-10,13-dimethyl-1,2,3,4,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[a] phenanthren-17-one	53-43-0	Natural hormone	DHEA
Dexamethasone	(8S,9R,10S,11S,13S,14S,16R,17R)-9- Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16- trimeth-yl-6,7,8,9,10,11,12,13,14,15,16,17- dodecahydro-3H-cyclopenta[a]phenanthren-3-one	50-02-2	Synthetic steroid	
Dibutyl phthalate	Di-n-butyl phthalate	84-74-2	Phthalate	DBP
Dibutyltin	Di-n-butyltin dichloride	683-18-1	Plastics stabilizer	DBT
Dicofol	2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethanol	115-32-2	Organochlorine Insecticide	
Dieldrin	(1aR,2R,2aS,3S,6R,6aR,7S,7aS)-3,4,5,6,9,9-Hexa-chloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-b]oxirene	60-57-1	Organochlorine Insecticide	
Diethyl hexyl phthalate	Bis(2-ethylhexyl)phthalate	117-81-7	Phthalate	DEHP
Mono-2-ethylhexyl phthalate	Phthalic acid mono-2-ethylhexyl ester	4376-20-9	DEHP Hydrolysis product	MEHP
Mono-n-butyl phthalate	Phthalic acid mono-2-n-butyl ester	131-70-4	DBP Hydrolysis product	MnBP
Diethylstilbestrol	4,4′-(3E)-Hex-3-ene-3,4-diyldiphenol	56-53-1	Synthetic estrogen	DES
Diisononyl phthalate	Bis(7-methyloctyl) phthalate	28553-12-0	Phthalate	DiNP
Diphenhydramine	2-(Diphenylmethoxy)-N,N-dimethylethanamine	58-73-1	Antihistamine	DPH
Dimethylbenz(a)anthra-cene	7,12-Dimethylbenz(a)anthracene	57-97-6	PAH	DMBA
Endosulfan (alpha/beta)	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro- 6,9-meth-ano-2,4,3-benzodioxathiepine-3-oxide	115-29-7	Organochlorine Insecticide	
Endrin	(1aR,2S,2aS,3S,6R,6aR,7R,7aS)-3,4,5,6,9,9-Hexa-chloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-b]oxirene	72-20-8	Organochlorine Insecticide	
Estradiol	17B-Estradiol, (17β)-estra-1,3,5(10)-triene-3,17-diol	50-28-2	Natural hormone	
Estrone	3-Hydroxy-13-methyl- 6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[a]phenanthren- 17- one	53-16-7	Natural hormone	E1
Ethinylestradiol	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol	57-63-6	Synthetic hormone	EE2
Ethylene thiourea	Imidazolidine-2-thione	96-45-7	Herbicide	

Ethylparaben	Ethyl 4-hydroxybenzoate	120-47-8	Antifungal Preservative	
Fadrozole	4-(5,6,7,8-Tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile	102676-31-3	Pharmaceutical	
Fenbuconazole	(RS)-4-(4-Chlorophenyl)-2-phenyl-2-(1H-1,2,4-triazol-1-ylmethyl)butyronitrile	114369-43-6	Fungicide	
Fenitrothion	O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate	122-14-5	Organophosphate Insecticide	
Fenoxycarb	Ethyl N-[2-(4-phenoxyphenoxy)ethyl]carbamate	72490-01-8	Insecticide	
Finasteride	N-(1,1-Dimethylethyl)-3-oxo-(5α,17β)-4-azaandrost-1-ene-17-carboxamide	98319-26-7	Pharmaceutical	
Fipronil	(RS)-5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile	120068-37-3	Insecticide	
Fluoxetine	(RS)-N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy] propan-1-amine	54910-89-3	Pharmaceutical	
Flutamide	2-Methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]-propanamide	13311-84-7	Pharmaceutical	
Fonofos	(RS)-(O-ethyl S-phenyl ethylphosphonodithioate)	944-22-9	Organophosphate Insecticide	
Formaldehyde	Methana, Formol, Methyl aldehyde, Methylene glycol	50-00-0	Solvent	
Furan	Oxole, Furfuran, 1,4-Epoxy-1,3-butadiene	110-00-9	Solvent	
Galaxolide	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-Hexamethylcyclopenta[g]-2-benzopyran	1222-05-5	Synthetic musk	HHCB
Genistein	5,7-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	446-72-0	Isoflavones, Phytestrogen	
Hexabromocyclododecane	1,2,5,6,9,10-Hexabromocyclododecane	25637-99-4	BFR	HBCDD
Hexachlorobenzene		118-74-1	Chlorinated Aromatic	HCB
Heptachlor	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene	76-44-8	Organochlorine Insecticide	
Heptachlor epoxide	1,4,5,6,7,8,8a-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene	1024-57-3	Organochlorine Insecticide Metabolite	
Hexachlorobutadiene	Hexachloro-1,3-butadiene	87-68-3	Solvent	
Heptachlorodibenzo-dioxin	eg 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	35822-46-9	Dioxin	HpCDD
HPTE	2,2-bis(4-hydroxyphenyl)-1,1,1-trichloroethane	2971-36-0	Methoxychlor Metabolite	
Iodine (I)	-	7553-56-2	Halogen; Essential element	
Kepone	Chlordecone; 1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-2H-1,3,4-(methanetriyl)cyclobuta[cd]pentalen-2-one	143-50-0	Organochlorine Insecticide	
Lead (Pb)	-	7439-92-1	Heavy metal	
Levonorgestrel	13-Ethyl-17-ethynyl-17-hydroxy- 1,2,6,7,8,9,10,11,12,13,14,15,16, 17- tetradecahydrocyclopenta[a] phenanthren-3-one	797-63-7	Synthetic Estrogen	
Lindane	gamma-Hexachlorocyclohexane	58-89-9	Organochlorine Insecticide	γ-HCH
Linuron	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea	330-55-2	Herbicide	
Malathion	Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate	121-75-5	Organophosphate Insecticide	
Mancozeb	Manganese ethylenebis(dithiocarbamate)	8018-01-7	herbicide	
Manganese		7439-96-5	Heavy metal	
Methylsulfonyl-DDE	3-Methylsulfonyl-2,2'-bis(4-chlorophenyl)-1,1'-dichloroethene	62938-14-1	DDE Metabolite	$MeSO_2$-DDE
Methoxychlor	2,2-Bis(4-methoxyphenyl)-1,1,1-trichloroethane	72-43-5	Organochlorine Insecticide	
Methyl bromide	Monobromomethane, 1-bromomethane	74-83-9	Fumigant, Pesticide	
Methyl farnesoate	Methyl (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienoate	10485-70-8	Juvenile Hormone	
Methyl triclosan	2,4-Dichloro-1-(4-chloro-2-methoxyphenoxy)benzene	4640-01-1	Triclosan Transformation Product	
Methylbenzylidene camphor	(3E)-1,7,7-Trimethyl-3-[(4-methylphenyl)methylene]-2-norbornanone	36861-47-9	UV filter	

Methylcholanthrene	20–Methylcholanthrene	56-49-5	PAH	
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1H-1,3,4-(methanetriyl)cyclobuta[cd]pentalene	2385-85-5	Organochlorine Insecticide	
Monosodium glutamate	Sodium 2-Aminopentanedioate	142-47-2	Food Additive	
n-Butylbenzene	n-Butylbenzene;Butylbenzene;1-Phenylbutane	104-51-8	Chemical Synthesis Intermediate	
Nicotine	3-[(2S)-1-Methylpyrrolidin-2-yl]pyridine	54-11-5	Alkaloid	
Nonachlor	trans-Nonachlor; (1S,7R)-1α,2β,3β,4,5,6,7,8,8-Nonachloro-2,3,3aα,4,7,7aα-hexahydro-4,7-methano-1H-indene	39765-80-5	Organochlorine Insecticide	
Nonylphenol	p-Nonylphenol; 4-Nonylphenol	104-40-5	Surfactant	NP
Norfluoxetine	Seproxetine; S)-3-phenyl-3-[4-(trifluoromethyl)phenoxy] propan-1-amine	126924-38-7	Pharmaceutical	
Octachlorodibenzo-p-dioxin	1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	3268-87-9	Dioxin	OCDD
Octachlorostyrene	1,2,3,4,5-Pentachloro-6-(trichlorovinyl)benzene	29082-74-4	Chlorinated Aromatic	OCS
Octyl-methoxycinnamate	2-Ethylhexyl (2E)-3-(4-methoxyphenyl)prop-2-enoate	5466-77-3	UV filter	
Octylphenol	p-Octylphenol; 4-n-Octylphenol	1806-26-4	Surfactant	OP
Oxychlordane	2-Endo,4,5,6,7,8,8-octachloro-2,3-exo-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-1-ex 3a,4,7,7a-tetrahydro-1,2-epoxy-4,5,6,7,8,8-hexachloro-4,7-methanoindan	27304-13-8	Chloridane Metabolite	
Parathion	O,O-Diethyl O-(4-nitrophenyl) phosphorothioate	56-38-2	Organophosphate Insecticide	
Pendimethalin	3,4-Dimethyl-2,6-dinitro-N-(1-ethylpropyl)aniline	40487-42-1	Herbicide	
Pentachlorobenzene	1,2,3,4,5-Pentachlorobenzene	608-93-5	Chlorinated Aromatic	
Pentachloronitrobenzene	2,3,4,5,6-Pentachloronitrobenzene; Quintozene	82-68-8	Herbicide	PCNB
Pentachlorophenol	2,3,4,5,6-Pentachlorophenol	87-86-5	Herbicide, fungicide	PCP
Perchlorate	Perchloric acid, ion(1-)	14797-73-0	Oxidizer	
Permethrin	3-Phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	52645-53-1	Insecticide	
PFDS	Perfluorodecanesulfonate/-sulfonic acid	67906-42-7, 335-77-3	PFAS	
PFHxS	Perfluorohexanesulfonate	108427-53-8	PFAS	
PFNA	Perfluorononanoate/-nonanoic acid	375-95-1	PFAS	
PFOA	Perfluorooctanoate/-octanoic acid	335-67-1	PFAS	
PFOS	Perfluorooctane sulfonate/-sulfonic acid	2795-39-3, 1763-23-1	PFAS	
PFOSF	Perfluorooctane sulfonyl fluoride	307-35-7	PFAS	
Phorate	O,O-Diethyl S-[(ethylsulfanyl)methyl] phosphorodithioate	298-02-2	Organophosphate Insecticide	
Picloram	4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid; Tordon 101	1918-02-1	Herbicide	
Polyvinylchloride	-	9002-86-2	Polymer; PVC	
8-Prenylnaringenin	(2S)-5,7-Dihydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-en-1-yl)-3,4-dihydro-2H-1-benzopyran-4-one; 8-Isopenenyl-naringenin	53846-50-7	Prenylflavonoid	
Procloraz	N-Propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]-1H-imidazole-1-carboxamide	67747-09-5	Fungicide	
Procymidone	3-(3,5-Dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0] hexane-2,4-dione	32809-16-8	Fungicide	
Prodiamine	5-Dipropylamino-α,α,α-trifluoro-4,6-dinitro-o-toluidine	29091-21-2	Herbicide	
Propylthiouracil	6-Propyl-2-sulfanylpyrimidin-4-one	51-52-5	Pharmaceutical	
Pyrene	Benzo[def]phenanthrene	129-00-0	PAH	
Pyrimethanil	4,6-Dimethyl-N-phenylpyrimidin-2-amine	53112-28-0	Fungicide	
Pyriproxyfen	4-Phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether	95737-68-1	Juvenile hormone	
Resorcinol	1,3-Dihydroxybenzene	108-46-3	Disinfectant, Chemical intermediate	
Sertraline	(1S,4S)-4-(3,4-Dichlorophenyl)-N-methyl-1,2,3,4-tetrahydro-naphthalen-1-amine	79617-96-2	Pharmaceutical	

Short chain chlorinated paraffins	Polychlorinated n-alkanes, C10 to C13, 50-60% chlorine	63449-39-8, 85535-84-8	Flame Retardant; plasticizer	SCCP
2,4,5-T	2,4,5 Trichlorophenoxyacetic acid	93-76-5	Herbicide	2,4,5,-T
Tamoxifen	(Z)-2-[4-(1,2-Diphenylbut-1-enyl)phenoxy]-N,N-dimethyle-thanamine	10540-29-1	Pharmaceutical	
Tetrabromobisphenol A	2,2′,6,6′-Tetrabromo-4,4′-isopropylidenediphenol	79-94-7	BFR	TBBPA
Testosterone	(8R,9S,10R,13S,14S,17S)- 17-Hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17- dodecahydrocyclopenta[a] phenanthren-3-one	58-22-0	Natural hormone	
Tetrachlorodibenzofuran	eg 2,3,7,8-Tetrachlorodibenzofuran	51207-31-9	Chlorinated dioxin	TCDF
Tetrachlorodibenzo-*p*-dioxin	eg 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6	Chlorinated dioxin	TCDD
PCB methyl sulfones	4-Methylsulfonyl-2,2′,3,4′,5′,6-hexachlorobiphenyl	116806-76-9	PCB metabolite	$MeSO_2$-PCB
Tetraiodothyronine	(2S)-2-Amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodo-phenyl]propanoic acid; Levothyroxine	51-48-9	Natural hormone	T4
Thiazopyr	Methyl 2-difluoromethyl-5-(4,5-dihydro-1,3-thiazol-2-yl)-4-isobutyl-6-trifluoromethylnicotinate	117718-60-2	Herbicide	
Toxaphene	Polychlorinated bornanes	8001-35-2	Organochlorine Insecticide	
2,4,6-Tribromophenol	1,3,5-Tribromo-2-hydroxybenzene	118-79-6	BFR, Natural product	2,4,6-TBP
Trenbolone	17β-Hydroxyestra-4,9,11-trien-3-one	10161-33-8	Anabolic Steroid	
Tributyltin	Bis(tri-n-butyltin)oxide	56-35-9	Fungicide	TBT
Trichloroethylene	1,1,2-Trichloroethene	79-01-6	Chlorinated Solvent	TCE
Trichlorophenate	2,4,5-Trichlorophenol; 2,4,6-Trichlorophenol; Trichlorophenol	95-95-4, 88-06-2	Fungicide	
Triclocarban	3-(4-Chlorophenyl)-1-(3,4-dichlorophenyl)urea	101-20-2	Microbicide	
Triclosan	5-Chloro-2-(2,4-dichlorophenoxy)phenol	3380-34-5	Microbicide	
Tri-iodothyronine	(2S)-2-Amino-3- [4-(4-hydroxy-3-iodo-phenoxy)- 3,5-diiodo-phenyl]propanoic acid	6893-02-3	Natural hormone	T3
Triphenyl phosphate	Phosphoric acid, triphenyl ester	115-86-6	Flame retardant	TPP
Triphenyltin	Fentin; (acetoxy)(triphenyl)stannane; hydroxytriphenylstan-nane	900-95-8, 668-34-8	Fungicide	
Venlafaxine	1-[2-Dimethylamino-1-(4-methoxyphenyl)-ethyl]cyclohexanol	93413-69-5	Pharmaceutical	
Vinclozolin	RS)-3-(3,5-Dichlorophenyl)-5-methyl- 5-vinyloxazolidine-2,4-dione	50471-44-8	Fungicide	
Zearalenone	(3S,11E)-14,16-Dihydroxy-3-methyl-3,4,5,6,9,10-hexa-hydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione	17924-92-4	Mycotoxins	

Abbreviations and Acronyms

AAARD	American Autoimmune Related Disease Association
ACC	Adrenocortical carcinoma
ACTH	Adrenocorticotropic hormone
AD	Autistic disorder
A.D.	Anno Domini
ADD	Attention deficit disorders
ADH	Antidiuretic hormone
ADHD	Attention deficit hyperactivity disorder
AGD	Anogenital distance
AhR	Aryl hydrocarbon receptor
AHS	Agricultural Health Study
AIDS	Acquired immunodeficiency syndrome
AITD	Autoimmune thyroid diseases
ALSPAC	Avon longitudinal study of parents and children
AMAP	Arctic Monitoring and Assessment Programme
AMH	Anti-Mullerian hormone
ANP	Atrial natriuetic peptide
ANSES	Agence national de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health and Safety)
AR	Androgen receptor
ART	Assisted reproduction techniques
ASRM	American Society for Reproductive Medicine
ATF	Activating transcription factor
ATP	Adenosine triphosphate
BCERC	Breast Cancer and the Environment Research Centers
BFRs	Brominated flame retardants
BMI	Body mass index
BMPs	Bone morphogenetic proteins
BPH	Benign prostatic hyperplasia
BSEF	Bromine Science and Environmental Forum
BW	Body weight
CAH	Congenital adrenal hyperplasia
cAMP	Cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CEBPs	CCAAT enhancer-binding proteins
CH	Congenital hypothyroidism
CHD	Coronary heart disease
CI	Confidence interval
CIS	Carcinoma *in situ*
COC	Combined oral contraceptives
COUP-TFII	Chicken ovalbumin upstream promoter-transcription factor II
COX-2	Cyclooxygenase-2
CPP	Central precocious puberty
CPs	Chlorinated paraffins
CRH	Corticotropin-releasing hormone
CRH-BP	Corticotropin-releasing hormone-binding protein
CT	Calcitonin
CVDs	Cardiovascular diseases
CYP	Cytochrome P
CXorf6	Chromosome X open reading frame
D1	Iodothyronine deiodinase type 1
D2	Iodothyronine deiodinase type 2
D3	Iodothyronine deiodinase type 3
DAX-1	Dosage-sensitive sex reversal, adrenal hypoplasia congenital, critical region on the X-chromosome, gene-1
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DIT	Developmental immunotoxicity
DMRT-1	Doublesex and mab-3 related transcription factor 1
DN	Disseminated neoplasia
DNA	Deoxyribonucleic acid
DOC	Deoxycorticosterone
E	Epinephrine
E1	Estrone
E2	Estradiol
EBAP	Environmental bioaccumulation potential
EC50	Half maximal effective concentration
EC90	90% maximal effective concentration
ECHA	European Chemicals Agency
ED	Endocrine disrupting
EDA	Effects directed analysis
EDCs	Endocrine disrupting chemicals
EDSP	Endocrine Disrupting Chemical Screening Program
EE2	Ethinylestradiol
EFSA	European Food Safety Authority
EGF	Epidermal growth factor
EPA	Environmental Protection Agency
EPO	Erythropoietin
ER	Estrogen receptors
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
EROD	Ethoxyresorufin o-deethylase
ERRγ	Estrogen related receptor gamma
ERT	Estrogen therapy
ESHRE	European Society of Human Reproduction and Embryology
esr-1 (ER alpha)	Estrogen receptor alpha
esr-2 (ER beta)	Estrogen receptor beta
ESPE	European Society for Pediatric Endocrinology
EU	European Union
FGF	Fibroblast growth factor
FGF 10	Fibroblast growth factor 10

FGF receptor 2	Fibroblast growth factor receptor 2
FSH	Follicle-stimulating hormone
FW	Fresh weight
GA	Gestational age
GABA	Gamma-aminobutyric acid
GC	Gas chromatography
GH	Growth hormone
GI	Gastrointestinal
GLOBOCAN	Cancer incidence and mortality worldwide
GLP	Good laboratory practice
GLP-1	Glucagon-like peptide-1
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptor
GRADE	Grading of recommendations assessment, development and evaluation
H295R	Human adrenocortical H295R cell line
HDL	High density lipoproteins
HELCOM	Helsinki Commission
HFA-DB	Health For All Database
HIV	Human immunodeficiency virus
HNF4A	Hepatocyte nuclear factor 4 alpha
Hox A10	Homeobox A10 gene
HOXA13	Homeobox A13 gene
HPA	Hypothalamic-pituitary-adrenal
HPCs	Halogenated phenolic chemicals
HPG	Hypothalamic-pituitary-gonadal
HPT	Hypothalamic-pituitary-thyroid
HRT	Hormone replacement therapeutics
HSD	Hydroxysteroid dehydrogenase
IARC	International Agency for Research on Cancer
IDDM	Insulin dependent diabetes mellitus
ICCM	International Conference on Chemicals Management
ICo	Intercollicular complex
IFCS	Intergovernmental Forum on Chemical Safety
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IGF	Insulin-like growth factor (IGF-1, IGF-II)
IgG	Immunoglobulin G
IGL	Internal granule layer
IgM	Immunoglobulin M
IKK	IκB kinase
IL-1	Interleukin-1
IL-4	Interleukin-4
IL-6	Interleukin-6
ILO	International Labour Organisation
IMT	Intimal medial thickness
INSL3	Insulin-like peptide 3
IPCC	Intergovernmental Panel on Climate Change
IPCS	International Programme on Chemical Safety
IQ	Intelligence quotient
IUCN	International Union for Conservation of Nature
IUGR	Intrauterine growth restriction
K_{OA}	Octanol-air partition coefficient
KOWIN	Model for prediction of octanol-water partition coefficients
K_{ow}	Octanol-water partition coefficient
KOAWIN	Model for prediction of octanol-air partition coefficients
LC	Leydig cell
LC	Liquid chromatography
LDL	Low density lipoproteins
LH	Luteinizing hormone
LIF	Leukemia inhibitory factor
LPI	Living planet index
LPD	Living planet database
LPUEs	Landings per unit effort
LXRs	Liver X receptors
MAMLD1	Mastermind-like domain containing 1
MAPK	Map kinase
MCF7	Breast cancer cell line Michigan cancer foundation
MCTB	Myobacterial copper transport protein B
mDCs	Myeloid dendritic cells
MHC-I	Major histocompatibility complex-class I
MIPS	Morphologically intermediate papilla syndrome
MOFs	Multi-oocyte follicles
MBP	Myeline basic protein
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MSH	Melanocyte-stimulating hormone
NAS	National Academy of Science
NCOA3	Nuclear receptor coactivator 3
NCHS	National Center for Health Statistics
NE	Norepinephrine
NHIS	National Health Interview Survey
NF-κB	Nuclear factor kappaB
NGO	Non-Governmental Organization
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIS	Sodium/iodide symporter
NK	Natural killer
NOAEL	No-observed-adverse-effect level
Nr4a1	Nuclear receptors 4a1
Nr4a3	Nuclear receptors 4a3
NRC	National Research Council
NR1I2	Nuclear receptor subfamily 1, group I, member 2

NSP-A	Neuroendocrine specific protein A
NYS	New York State
OATP	Organic anion-transporting polypeptide
OCs	Organochlorines
OECD	Organization for Economic Cooperation and Development
OVA	Ovalbumin
OSPAR	Oslo/Paris convention
PAC	Polycyclic aromatic compounds
PACE	Partnership for Action on Computing Equipment
PAHs	Polycyclic aromatic hydrocarbons
PCOS	Polycystic ovary syndrome
PES	Pediatric Endocrine Society
PFCs	Perfluorinated compounds
PGCs	Primordial germ cells
PGE2	Prostaglandin E2
PM2.5	Particulate matter 2.5
POF	Premature ovarian failure
POPs	Persistent organic pollutants
PPAR	Peroxisome proliferator-activated receptors
PPARα	Peroxisome proliferator-activated receptor alpha
PPAR(gamma)	Peroxisome proliferator-activated receptor gamma
PPARG	Peroxisome proliferator-activated receptor gamma
PPARγ	Proliferator-activated receptor gamma
PR	Progesterone receptor
PRL	Prolactin
PROD	Penthyloxyresorufin o-depenthylase
PROS	Pediatric Research in Office Settings
PSA	Prostate-specific antigen
PTH	Parathyroid hormone
PXR	Pregnane X receptor
PYY	Pancreatic peptide YY
QSAR	Quantitative structure-activity relationship
RC 3	Rat cortex clone 3
REACH	Registration, evaluation, authorization and restriction of chemicals
RfD	Reference dose
RLI	Red list index
ROPME	Regional Organization for the Protection of the Marine Environment
ROR	Retinoid-related orphan receptor
ROS	Reactive oxygen species
RXFP2	Relaxin/insulin-like family peptide receptor 2
RXR	Retinoid-X receptor
SAICM	Strategic Approach to International Cemicals Management
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SGA	Small for gestational age
Shh	Sonic hedgehog
SOLEC	State of the lakes ecosystem conference
SPE	Solid phase extraction
SRKW	Southern resident killer whales
SSRIs	Selective serotonin reuptake inhibitors
StAR	Steroidogenic acute regulatory
STAT3	Signal transducer and activator of transcription 3
STW	Sewage treatment works
SXR	Steroid and xenobiotic receptor
T	Testosterone
T1DM	Type 1 diabetes mellitus
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine binding globulin
TDI	Tolerable daily intake
TDS	Testicular dysgenesis syndrome
TEDX	The endocrine disruption exchange
TEQ	Toxic equivalent
TGC	Testicular germ cell cancers
TH	Thyroid hormone
Th2	T helper cell 2
TNFα	Tumor necrosis factor alpha
TPs	Transformation products
TPO	Thyroperoxidase
TR	Thyroid hormone receptor
TRα	Thyroid hormone receptor alpha
TRβ	Thyroid hormone receptor beta
TRH	Thyrotropin-releasing hormone
TSCA	Toxic substances control act
TSH	Thyroid-stimulating hormone
TTR	Transthyretin
UDPGT	Uridine diphosphate glucuronyltransferase
UGP	Urogenital papillae
UK	United Kingdom
UN	United Nations
UNEP	United Nations Environment Programme
US	United States
USA	United States of America
US EPA	United States Environmental Protection Agency
US NAS	United States National Academy of Sciences
US NTP	US National Toxicology Program
UV	Ultraviolet
VTG	Vitellogenin
WBI	Wild bird index
WHO	World Health Organization
WOE	Weight of evidence
WPSI	Water bird population status index

Endocrine Disrupting Chemicals have many sources

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