2.1 General Background

Since the publication of Rachel Carson's *Silent Spring* (Carson, 1962), there has been increasing awareness that chemicals in the environment can exert profound and deleterious effects on wildlife populations and that human health is inextricably linked to the health of the environment. The last two decades, in particular, have witnessed a growing scientific concern, public debate, and media attention over the possible deleterious effects in humans and wildlife that may result from exposure to chemicals that have the potential to interfere with the endocrine system. The intensity of the concerns and lack of consensus among scientists can best be ameliorated by an objective evaluation of the available scientific data on the potential adverse effects of these chemicals from a global perspective. Countries lacking the necessary infrastructure to monitor and evaluate these chemicals expressed a particular need for an objective international assessment. The document builds on existing assessment documents and reviews (see Table 2.1) and is not intended as a thorough, comprehensive literature review. Only peer-reviewed literature or publicly available reports were evaluated. It is **not** a risk assessment or consensus document. Neither is it an assessment of available test methodologies for detecting EDCs. Both the OECD and a number of national organizations are addressing these issues (ECETOC, 1996; OECD, 1998a, 1998b, 1999a; US EPA, 1998a; Kanno et al., 2000).

EDCs encompass a variety of chemical classes, including natural and synthetic hormones, plant constituents, pesticides, compounds used in the plastics industry and in consumer products, and other industrial by-products and pollutants. They are often pervasive and widely dispersed in the environment. Some are persistent, can be transported long distances across national boundaries, and have been found in virtually all regions of the world. Others are rapidly degraded in the environment or human body or may be present for only short periods of time but at critical periods of development.

List of Abbreviations

AhR Aryl hydrocarbon receptor
DDE Dichlorodiphenyl dichloroethylene
DDT Dichlorodiphenyl trichloroethane
EDCs Endocrine-disrupting chemicals
ECETOC European Centre for Ecotoxicology and Toxicology of Chemicals
ER Estrogen receptor (α and β isoforms)
IUPAC International Union of Pure and Applied Chemistry
JEA Japan Environment Agency
LH Luteinizing hormone
MRC Medical Research Council
NRC National Research Council
NTP National Toxicology Program
OECD Organisation for Economic Co-operation and Development
PCBs Polychlorinated biphenyls
PCDDs Polychlorinated dibenzodioxins
PCDFs Polychlorinated dibenzofurans
POPs Persistent organic pollutants
SETAC Society for Environmental Toxicology and Chemistry
TCDD 2,3,7,8-Tetrachlorodibenzyl-*p*-dioxin
UBA Umweltbundesamt (German Environmental Agency)
UNEP United Nations Environment Programme
US EPA United States Environmental Protection Agency

2.2 Generic Issues

There are a number of complex issues that must be considered when evaluating the effects of endocrine disruptors (Ashby et al., 1997b; Ashby, 2000). These are summarized in this chapter and are discussed in detail in subsequent chapters (detailed references are also provided in subsequent chapters). Studies that clearly address exposure–outcome relationships are the most valuable in assessing the impact of EDCs on wildlife and human health. Unfortunately, many of the epidemiological or wildlife studies do not have good measures of exposure, which limits our ability to draw firm conclusions from them. This problem is especially prevalent for those EDCs that are rapidly degraded in the environment or in the human body. This means that the exposure that might have caused an adverse outcome (e.g., a reproductive deficit) is not detectable at the time that clinical manifestations become evident. For this reason, most of the EDCs that are relied on to draw cause-and-effect relationships are those that are biologically and ecologically persistent (e.g., PCBs, DDT, and dioxins). A number of these POPs are known to endanger human health and ecosystems and have been the subject of global conventions. Twelve POPs were singled out for elimination and/or reduction in a legally binding global treaty, which was signed by 115 countries in Stockholm in May 2001. These initial 12 high-priority POPs were selected based on data that demonstrate adverse exposure–outcome relationships in humans and wildlife, and processes are being implemented to add additional chemicals to the list.

This document emphasizes those chemical exposures and adverse human and ecological outcomes where an indication of cause and effect via multiple mechanisms of endocrine disruption has been demonstrated or hypothesized. These case studies also illustrate the types of interferences that are of importance and the variety of adverse health outcomes that can be exhibited.

One of the major issues that needs to be addressed when evaluating the impact of EDCs on human health and the environment is whether effects reported in the literature represent isolated cases or more global responses. For example, diminished wildlife populations adjacent to a significant point source may not be indicative of global responses. In contrast, relatively small effects on wildlife or human health points might have great impact if those responses are global in nature. Another problem in assessing the health impacts of EDCs is that some of these chemicals have been shown to contribute to the incidence of common diseases of multifactorial etiology (e.g., infertility, cancer, neurobehavioral deficits). Therefore, it will be difficult to attribute effects in traditional epidemiology studies to EDCs unless those effects are seen in large numbers of people.

2.3 Mechanisms of Endocrine Disruption in Humans and Wildlife

There are a number of mechanisms whereby EDCs can modulate endocrine systems and potentially cause adverse effects (see also Chapter 3). The generally accepted paradigm for receptor-mediated responses includes binding of hormone to its receptor at the cell surface, cytoplasm, or nucleus, followed by a complex series of events that lead to changes in gene expression characteristic for a specific hormone (Birnbaum, 1994). It is thought that changes in gene expression represent an early but critical step in the regulation

Table 2.1 - Selected Workshop/Committee/Assessment Reports on Endocrine Disruption

Year	Organization	Purpose/Scope	Reference
1992	World Wildlife Fund	Examination of the commonalities of adverse effects in wildlife, experimental animals, and humans. Produced the "Wingspread Consensus Statement"	Colborn and Clement, 1992
1994	National Institute of Environmental Health Sciences	Review of cellular biology, developmental effects, sources, and global health implications of environmental estrogens	Maclachlan and Korach, 1995
1995	German Federal Environmental Agency	Discussion about the occurrence and impact of endocrine disruptors and the potential risks that may arise to humans and the environment	German Federal Environment Agency, 1996
1995	Ministry of Environment and Energy, Denmark	Evaluation of the effects of estrogens on male reproductive development and function	Toppari et al., 1996
1995	US EPA	Workshop on research needs for the Risk Assessment of health and environmental effects of endocrine disruptors (April 1995)	Kavlock et al., 1996
1995	Chemical Manufacturer's Association; World Wildlife Fund; US EPA	Workshop on screening methods for chemicals that alter thyroid hormone homeostasis, action, and function	Ankley et al., 1998a
1995	UK Medical Research Council Institute for Environment & Health	Assessment on Environmental Estrogens: Consequences to Human Health and Wildlife	MRC Institute for Environment and Health, 1995
1996	European Commission	European workshop on the impact of endocrine disruptors on human health and wildlife, Wyebridge U.K.	European Commission, 1996
1996	ECETOC	Compendium of test methods for environmental estrogens	ECETOC, 1996
1996	SETAC	Workshop on principles and processes for evaluating endocrine disruption in wildlife	Kendall et al., 1998
1996	US Committee on the Environment and Natural Resources	Development of a national planning framework for endocrine disruptor research and analysis of the existing federally funded research projects to help identify information gaps	Reiter et al., 1998
1996	US EPA	Workshop on the development of a risk strategy for assessing the ecological risk of endocrine disruption	Ankley et al., 1997
1997	UNEP, US EPA, White House Office of Science and Technology; Alton Jones Foundation	International workshop on endocrine disruptors	UNEP, 1997
1997	Federal Environment Agency, Germany	Workshop on effects of endocrine disruptors on neuronal development and behavior	UBA, 1997
1997	SETAC, OECD, European Commission	Expert Workshop on Endocrine Modulators and Wildlife: Assessment and Testing	SETAC, 1997
1997	US EPA	Special report on environmental endocrine disruption: an effects assessment and analysis	US EPA, 1997
1997	OECD	Critical assessment of the ability of existing OECD test methods to detect sex hormones disrupting potential	OECD, 1997
1997	Health Council of the Netherlands	Evaluation of the effects of endocrine disruptors on human reproduction and development	Health Council, Netherlands, 1997
1997	International Life Sciences Institute; US EPA	Scientific evaluation of the potential for substances in the diet to influence the human endocrine system	ILSI, 1998
1997	Japan Chemical Industry Association	Evaluation of status and research needs of endocrine disrupting compounds in Japan	Japan Chemical Industry Association, 1997
1998	Swedish Environmental Protection Agency	Endocrine disrupting substances-impairment of reproduction and development	Olsson et al., 1998
1998	International Union of Pharmacology	Natural and anthropogenic environmental estrogens—the scientific basis for risk assessment	IUPAC, 1998
1998	Society of Environmental Toxicology and Chemistry	Workshop on endocrine disruption in invertebrates	DeFur et al., 1999
1999	National Research Council	Hormonally active agents in the environment	NRC, 1999
1999	European Commission	Scientific committee on toxicity, ecotoxicity, and the environment opinion on human and wildlife health effects of endocrine disruptors	Vos et al., 2000
2000	Health and Environment Canada	Workshop on endocrine disrupting substances in the Canadian environment	Servos and Van Der Kraak, 2001
2000	US National Toxicology Program	Report of the endocrine disruptors low-dose peer review	NTP, 2001a
2000	Finnish Environment Institute	Research for Management of Environmental Risks from Endocrine Disruptors	Assmuth and Louekari, 2000
2001	Federal Environment Agency, Germany	Second status seminar on endocrine disruptors	UBA, 2001a

of normal biological function, including cell proliferation and differentiation responses essential for normal development and the function of multiple organ systems. Although there is considerable information on the early molecular events involved in hormone response, there is very little knowledge concerning the relationship between those molecular events and adverse health effects such as cancer or reproductive toxicity. This knowledge gap is perhaps the most limiting factor in our ability to evaluate exposure–response relationships, particularly following low-level exposure to potential EDCs. The use of new approaches in molecular epidemiology and animal model systems has the potential to yield additional valuable information for elucidating the role of these mechanistic determinants of specificity at low-dose exposures to potential EDCs and for improved risk evaluations for the adverse health effects of EDCs. There are numerous experimental systems available for evaluating the interactions of exogenous or synthetic chemicals with hormone systems, particularly those that interact with the estrogen, androgen, thyroid, and AhRs (Bolander, 1994). However, there is a growing body of knowledge that interactions of chemicals with other receptor systems also may be important in the EDC arena. These include the retinoic acid receptor, cytokine systems, and a number of so-called orphan receptors (receptors without unknown ligands and/or functions) such as the peroxisome proliferator receptor system. In general, these receptor systems are remarkably well conserved phylogenetically, suggesting that data from wildlife and experimental models should be useful, although not necessarily definitive, for estimating risks from EDC exposure to humans.

The mechanism or mode of action of EDCs is not limited to those agents that interact directly with hormone receptors. Other mechanisms of interest include inhibition of hormone synthesis, transport, or metabolism and activation of receptor through processors such as receptor phosphorylation or the release of cellular complexes necessary for hormone action. In the case of hormone synthesis, considerable research has been conducted on the aromatase inhibitors that prevent the conversion of androgens to estrogens through a cytochrome P450 system that is highly conserved in many species. Several fungicides have been shown to cause infertility by aromatase inhibition. In addition, there is a growing awareness that multiple receptor systems act in concert to regulate biological functions. For example, "cross talk" between the ER and growth factor receptors appears necessary for estrogen signaling of mammary cells to divide or differentiate. These events are critical for explaining several risk factors for breast cancer such as age at menarche, age at menopause, or effects of numbers of pregnancies. There are numerous other kinds of cross talk between various constituents of the endocrine system, and an understanding of the mechanisms involved could improve our ability to produce credible health assessments of EDCs. One well-known example of "cross talk" involves antiandrogen-mediated elevation of endogenous estrogen levels due to increased LH production.

Other examples of EDCs capable of acting at multiple cellular sites via multiple endocrine mechanisms are discussed in detail in Chapter 3. For example, the pesticide methoxychlor displays estrogen agonist (ER-β) activity because some of its metabolites bind to the ER. It also possesses antiandrogenic actions through a more poorly defined mechanism involving the hypothalamic-pituitary-gonadal axis. Another example is the ability of the DDT metabolite DDE to act as an antiandrogen by inhibiting testosterone binding with the androgen receptor, but these antiandrogenic effects may also be facilitated by the effects of DDE on steroid-metabolizing enzyme expression.

There are many factors that should be considered when utilizing mechanistic information on EDCs in health assessments. Of particular concern are species, interindividual, and tissue specificity in endocrine signaling pathways. Differential responsiveness to EDCs has been observed between different species and extends to interindividual differences within a species and between different tissues as well. The biologic and molecular mechanisms underlying this specificity are quite diverse. Determinants of species specificity include differences that exist between species in receptor binding, gene transcription patterns of gene expression, and cellular responses to endocrine-active compounds. Interindividual differences in responsiveness may be determined at the level of genetic polymorphisms in hormone-metabolizing enzymes, hormone receptors, and those genes that are activated by these receptors. Our rapidly growing knowledge base emerging from the human genome project will enable the design of rational studies on the impact of EDC exposures on hormonally sensitive end points in groups that may be genetically predisposed. Extrinsic factors such as diet can also impact individual susceptibility to endocrine-active agents.

The organochlorine chemicals provide interesting insights regarding the mechanisms of action for EDCs. In the case of the dioxins, the scientific consensus is that most, if not all, of their effects require an initial interaction with a cellular protein termed AhR (Poland and Glover, 1977). As discussed in Chapter 3, the ligand-bound AhR is capable of interacting with a number of critical signal transduction pathways, leading, for example, to involvement with xenobiotic metabolizing enzymes, steroid receptor signaling, growth factor expression, circadian clocks, responses to hypoxia, and angiogenesis. Through these varied interactions, such chemicals are able to induce a wide spectrum of biological effects at a number of different life stages in a variety of species, and some of these responses do not easily fit the traditional definition of endocrine-mediated events. Although adverse biological effects mediated via the activation of AhR are certainly of concern, a higher burden of proof is needed to identify such effects as due to endocrine disruption than merely the correlation between ability to bind to the receptor and the elicitation of some biological effect. In this regard, the considerations detailed in section 3.16 were used in evaluating the extent to which information would be included in this document. Although information on the involvement of the AhR in wildlife is limited, in our final conclusions on the strength of the evidence for relationships between chemicals and endocrine-mediated effects, the same criteria were applied for both humans and wildlife.

2.4 Dose–Response Relationships

The issue of dose–response relationships is perhaps the most controversial issue regarding EDCs. One of the reasons is that EDCs often act by mimicking or antagonizing the actions of naturally occurring hormones. These hormones are already at physiologically functional concentrations, so the dose–response considerations for EDCs are often different than for other chemicals that are not acting directly on the endocrine system. Reported low-dose effects for EDCs have come under intense scrutiny regarding the question of the adequacy of traditional toxicology testing paradigms for detecting low-dose effects. A recent workshop on this issue (NTP, 2001a) concluded that although low-dose effects may be occurring, those effects often are not replicated

consistently, and the toxicological significance of the reported effects is not known. Dose–response issues should be explicitly considered when studies are designed for risk evaluation for health or wildlife effects. Of particular relevance is the issue of dose selection. Ideally, the doses used should span a wide range to identify both toxic and mechanistic end points. The issue of dose selection has become critical to the current controversies surrounding the issue of biphasic dose–response curves for EDC effects on end points such as prostate weight. Although there may never be complete knowledge on the mechanism(s) of action for any chemical, some knowledge of key events could help clarify dose–response relationships.

Timing of exposure is also critical to the understanding of dose–response relationships for EDCs. Numerous examples exist in the literature where age at exposure is a known risk factor. For example, endocrine disruption of the developing brain can permanently alter behavior, whereas similar exposures to a fully differentiated brain could be without effect. Ecological and wildlife effects are also strongly influenced by the timing of exposure (e.g., during the breeding season).

Population heterogeneity is another important factor in dose–response evaluation. For human health, a number of factors contribute to a wide range of risks, including genetic predisposition, age, gender, diet, disease conditions, and past exposures. The range of risk modulators may be even greater for complex ecosystems, but little information is available in this area.

Evaluation of the dose–response relationships for health and environmental effects of endocrine disruptors will be most credible when information is available from several sources (e.g., toxicity studies, mechanistic and epidemiological studies, and field studies). There are a number of issues that are helpful to consider when embarking on dose–response assessment. These include, but are not limited to, 1) the adequacy of relevant experimental models for evaluating potential human effects of low-dose exposure to endocrine disruptors, 2) state of knowledge concerning quantitative relationships among the various processes maintaining homeostasis for the tissue, organ, or function being studied, 3) how perturbations in homeostasis lead to disease or dysfunction, 4) whether these changes can be quantified, 5) an understanding of the mechanisms through which endocrine disruptors perturb homeostasis and endocrine function and alter the risks from that of normal levels of endogenous hormones, 6) consideration of how differences in lifestyle factors (diet, nutrition, etc.) affect sensitivity to endocrine disruption, 7) an understanding of how the age of the endocrine system alters sensitivity to endocrine disruption, and 8) how interindividual differences (based on genetic variation) in constituents of endocrine pathways (e.g., receptor variants) alter responses to endocrine-sensitive end points caused by exposure to EDCs. Most of these considerations are relevant to both human and wildlife effects of EDCs.

A common dose–response relationship for all effects and for all endocrine disruption mechanisms should not be expected. This conclusion is based on the knowledge that there are many different kinds of hormonal actions of chemicals categorized as endocrine disruptors. These activities include estrogenic, antiestrogenic, antiandrogenic, growth factor modulation, cytokine and thyroid modulation, modulation of hormone metabolism, among many others.

2.5 Exposure Issues

There are numerous chemicals in the environment (e.g., pesticides, industrial chemicals, and natural products) that are hormonally active, and these can be detected in people and wildlife as well as in environmental samples. Some of these persist in the environment and others do not. Some are lipophilic, sequestered in adipose tissue and secreted in milk, and others may only be present for short periods of time but at critical periods of development. Our knowledge about the magnitude of human or wildlife exposure remains very limited. Most of the more definitive studies on chemically mediated effects, including those on EDCs, have been conducted on highly exposed groups in various occupations or from accidental exposures. In only a few cases, appropriate exposure information is available from lower level environmental exposures because of analytical sensitivity and the latency in outcome after exposure has occurred.

Hormonally active environmental chemicals are extraordinarily diverse in their structure and potency. For example, some organohalogens, such as the PCBs, DDT, PCDDs, and PCDFs, are suspected endocrine-disrupting agents, but various members of these groups of chemicals exhibit profound differences in potency, biological and ecological persistence, and mechanisms of action. For example, 75 PCDD congeners and 135 PCDF congeners vary tremendously in their potency to exhibit TCDD-like activity (see Chapter 6). This kind of diversity creates obvious problems in human and ecological health assessments, and it also increases the complexity and costs of analyzing for concentrations of these chemicals in biological samples. In addition to the PCBs, PCDDs, and PCDFs, many other kinds of chemical modulators of endocrine function are examined in this report. These include phthalate acid esters, DDT and DDE, alkylphenols, methoxychlor, bisphenol, diethylstilbestrol, estradiol as an ecological contaminant, the fungicide vinclozolin, and several other synthetic chemicals that are reported to interact with various components of the endocrine system.

Synthetic chemicals are not the only exogenous agents that have caused health concerns because of their hormonelike activity. Of particular interest are the phytoestrogens (such as genistein and equol) and the fungal estrogens (such as zearalenone). The phytoestrogens and fungal estrogens are diverse in structure, undergo complex metabolic processes, and are ubiquitous in the environment. They can be found in blood and urine samples of virtually every person and animal on this planet, often in high concentrations. They pose difficult analytical issues, yet if exposure–response relationships for the phytoestrogens remain uncertain, health assessments for many endocrine-disrupting agents, particularly the environmental estrogens, will also remain uncertain. This is because several of the phytoestrogens, mostly notably genistein and its analogs, possess binding affinities for the ER far greater than many of the EDCs of concern, such as the alkylphenols, bisphenol A, and DDE. From a potency standpoint, the phytoestrogens exert a far greater impact on human exposure to exogenous estrogens than do the synthetic chemicals. This does not mean that we should not be concerned about synthetic estrogens, but it does emphasize that exposure assessments for EDCs need to consider both the magnitude of exposure and relative potencies of the array of EDCs that may be encountered in the home, workplace, and general environment.

Information is needed to more accurately quantify the human, wildlife, and environmental burden of hormonally active environmental chemicals so that quantitative comparisons can be made between body levels of natural and exogenous hormones based on potency, not just absolute amount. This kind of information is essential if we ever hope to properly evaluate

exposure–response relationships in field and epidemiology studies and to use those relationships to produce credible risk assessments. Data on historical and geographic trends of exposure to EDCs are generally lacking. Knowledge of the fate and transport of new and existing chemicals is also limited, particularly among the different environmental compartments (water, sediment, and biota).

Exposure assessment, particularly as it involves human health, must focus on vulnerable groups, in terms of both life stage and lifestyle. Exposure assessment for the critical development stages remains a high research priority. These stages include gestation, lactation, adolescence, and senescence. The endocrine system, through a developmentally regulated pattern of expression, controls the pathways essential for cell proliferation, differentiation, and organ development, so it is not surprising that perturbations of the endocrine system during critical windows of sensitivity create the greatest potential for adverse health effects.

Vulnerability of different groups in the population will be affected by lifestyle factors (e.g., subsistence hunting and fishing, and avid sportsmen who consume fish and wildlife), genetic factors (e.g., metabolic differences that can determine sensitivity), special dietary habits, and age (e.g., the types and rates of food consumption in children). Although there is general agreement that diet would likely be the major exposure route for exposure to the EDCs, an approach based on integrated exposure assessment needs to be taken. All routes should be examined (e.g., dermal, inhalation, and ingestion). Examining the exposure of humans or wildlife to multiple chemicals (especially for chemicals with a common mode of action and/or common target sites) that may function as EDCs is also critical.

Exposure assessment encompasses both external measurements (levels in air, water, soil, food, etc.) and internal measurements (levels in blood, urine, and tissue samples). Both kinds of measurements provide critical information for wildlife, epidemiological, and experimental studies. Internal measurements are often confounded by the rapid metabolism of some EDCs (Elsby et al., 2001). This means that quantification of metabolites or degradation products in biological samples is necessary for endocrine disruptor research. Some of the rapidly metabolized chemicals reviewed in this document are the phthalate acid esters, alkylphenols, diethylstilbestrol, some PCBs, phytoestrogens, and methoxychlor.

Other complications in exposure assessment include time lags, seasonality, and multiple chemical exposures. a) Time lags between exposure and effect: The transgenerational nature of some EDC effects may be the single most complicating factor. All of the potential latent effects that may occur from short-term exposures during critical development windows have not yet been identified. b) Seasonality: Because of the sensitivity of reproductive stages to EDCs, seasonality will be extremely important to wildlife. In addition, the association of EDCs with the aquatic environment is complicated by seasonal rainfall, storm runoff, and water releases. c) Multiple chemical exposures: This, too, is a factor for any toxic chemical, but it is especially identified here because of the potential for effect modification (e.g., synergy, additivity, or antagonism).

The most critical need on status and trends is for the continuation and improvement of monitoring of the environment for the presence and magnitude of contaminants. Although environmental and tissue levels of certain EDCs (e.g., PCBs) have declined in some countries in response to regulations, they remain of concern in other countries, and uncertainty still exists regarding future trends. For most EDCs, data on trends are not available. Long-term data using harmonized collection and analysis methods are needed. Existing programs that furnish repeated measures of chemical contamination in the environment or in food provide our only indication of whether exposure is increasing or decreasing, and to what magnitude.