Endocrine disrupting substances

– Impairment of reproduction and development

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Cover picture:
The moorfrog male (***Rana arvalis***) turns blue in the mating season
– an example of naturally induced effects by hormones.
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PREFACE

The Scientific Committee for Toxicology and Environmental Health at the Swedish Environmental Protection Agency early brought up the question regarding hazards related to environmental chemicals with hormone disrupting properties. It was decided to make an up to date review to be used by Swedish authorities and as a basis for a research programme.

The present report gives a basis for the differences in sexual development between different organisms and the role of hormones. The aim is to explain the role of foreign chemicals with regard to endocrine disrupting properties in different organisms. Suggested areas for further research have also been included.

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Contents

SVENSK SAMMANFATTNING

Den föreliggande rapporten avser att ge en sammanfattning av det nuvarande kunskapsläget vad gäller kända och befarade störningar av fortplantning hos ryggradsdjur. En utvärdering av möjliga samband mellan iakttagna effekter, på både djur och människor, och kemikalier som släpps ut i miljön har gjorts. Rekommendationer för framtida forskning, med tonvikt på svenska problem, ges i slutet av rapporten.

Det befaras att ett stort antal olika substanser, som släpps ut i miljön, kan påverka hormonsystem och därigenom störa fortplantning och utveckling hos ryggradsdjur. Att ett samband skulle föreligga mellan störd fortplantning och utsläpp av kemikalier i miljön har indikerats i flera studier. Detta har lett till att ett flertal utredningar har genomförts för att sammanställa information om östrogen aktivitet hos kemikalier i miljön. Förutom östrogena system kan dock även andra hormonsystem påverkas och effekter har iakttagits på andra könshormonsystem, som androgener och progestiner, men även på hormoner som thyroxiner och retinoider. I många fall är det samma grupper av kemikalier som påverkar dessa olika hormonsystem. Flertalet av de substanser som befaras ha endokrint störande effekter omvandlas via en enzymfamilj kallad cytokrom P450 (CYP). CYP är inblandat i bildning och omvandling av steroider men är även viktig för avgiftning av främmande substanser. Genom att CYP ändrar strukturen hos substanser finns även möjligheten att en substans omvandlas från att påverka ett hormonsystem till att påverka ett annat. Komplexiteten i effekter som kan uppstå som ett resultat av exponering för en enskild substans gör att tolkningen av dess interaktioner med ett enskilt hormonsystem försvåras. Det är därför nödvändigt att rikta uppmärksamheten på ett flertal hormonsystem.

RAPPORTENS MÅL

Rapporten är i huvudsak fokuserad på kemikalier som, genom att verka som agonister eller antagonister, kan befaras störa hormonell signalering hos ryggradsdjur. Förutom iakttagelser av imposex (partiell hermafrodism hos honor) har inte effekter på ryggradslösa djur tagits med i utredningen. Väldigt lite är känt vad gäller miljögifters effekter på fortplantning hos ryggradslösa djur. Hormoner är inblandade i alla utvecklingsstadier hos ryggradslösa djur och avsaknaden av information om effekter på dessa organismer kan bero på avsaknad av studier på dessa och betyder därför inte att de inte kan påverkas. Vi bedömer därför att detta område snarare har varit negligerat än att det inte föreligger effekter.

Definition av endokrint störande ämnen (endocrine disrupting substances; EDS)

Det är nödvändigt att definiera vad som utgör en endokrint störande substans. Vid ett europeiskt möte angående ”impact of endocrine disrupters on human health and wildlife”, som hölls i december 1996 i Weybridge, UK gavs denna definition av EDS ”Ett endokrint störande ämne är en kroppsfrämmande substans som orsakar kritiska hälsoeffekter i en intakt organism, eller dess avkomma, som en konsekvens av ändringar i endokrin funktion”. Det definierades även att ”en *potentiell* EDS är en substans som har egenskaper som kan befaras leda till endokrina störningar i en intakt organism”. Som dessa definitioner föreslår så är det viktigt att utveckla *in vivo* system för att klassificera substanser som varande EDS medan *in vitro* system är viktiga för att välja ut substanser som varande möjliga EDS. I denna rapport använder vi oss av dessa definitioner.

Rapportens innehåll

Hormoner är inblandade i regleringen av många olika och viktiga processer hos organismer. Till dessa hör reglering av fortplantning, utveckling och ämnesomsättning. Många kemiska ämnen kan störa dessa funktioner genom att påverka de hormonella systemen. För att förstå hur hormonsystemen störs krävs det kunskap om deras naturliga funktioner Utredningen har fokuserat på tre centrala hormonsystem, könshormoner (östrogener, androgener och progestiner), thyroidhormon och retinoider. Dessa beskrivs i kapitel 2.

Könsbestämningen hos många ryggradsdjur styrs via könshormonerna. Detta gäller främst fiskar och kräldjur. Detta kan leda till att kemikalier i miljön kan påverka könskvoterna hos dessa djur. Utvecklingen av könsorgan och könsbeteende styrs av könshormon även hos människa. I kapitel 3 görs en genomgång av mekanismer för könsbestämning och utveckling hos ryggradsdjur.

En stort antal kemikalier befaras ge effekter på fortplantning och utveckling genom att störa hormonsystem som könshormoner, thyroidhormon och retinoider. I kapitel 4 görs en genomgång av olika iakttagna och befarade effekter på människor och djur. Därefter görs en genomgång i kapitel 5 av olika kemikalier som befaras ha hormonella effekter. Ett mångfald av experiment har utförts på olika organismer för att utröna hur olika kemiska ämnen påverkar de olika hormonsystemen. I kapitel 6 görs en genomgång av experimentella studier som har gjorts på dessa kemikalier. I kapitel 7 ges en utvärdering av de iakttagna effekterna och rekommendationer för fortsatta studier.

ENGLISH SUMMARY

There is a current concern that an increasing number of substances, with possible endocrine disrupting potential, are being released into the environment and that these substances may have deleterious effects on vertebrate reproduction. Possible links between disturbed reproduction and exposure to environmental chemicals have been suggested in different studies. There are several international reports which address the estrogenic activity of such chemicals. Besides the estrogen system, many other endocrine systems may be affected by chemicals released into the environment. Thus, effects have also been observed on other steroid systems, such as androgens and progestins, as well as on the thyroid and retinoid systems. In many instances several of these systems may be affected by the same groups of chemicals. Metabolism of many of these chemicals is controlled by different forms of cytochrome P450, and altered structure, due to metabolism of the chemical, may redirect the effect from one system to another. The complexity of effects which may be the result of exposure to any one substance render the interpretation of its interaction with any single system difficult.

THE OBJECTIVES OF THE REPORT

The present report aims to give an up to date account of the literature on known and suspected disturbances of reproduction in vertebrates and to examine the possible association between observed effects, in both humans and wild-life, and chemicals that are released into the environment, with special emphasis on Swedish conditions. Following the evaluation of existing knowledge, recommendations for future research are given.

In the present report we are primarily focusing on chemicals that have the potential of interfering, as agonists or antagonists, with hormonal signaling in vertebrates. We have not included effects on evertebrates, except for the development of imposex. Today there is very little known about the reproductive impairment of pollutants in arthropoda. This may be a reflection of the lack of studies on these organisms rather than that effects do not exist. It is known that the endocrine system plays important roles in all stages of development of arthropoda (e.g. growth, ecdysis, hibernation, sexual maturation) and that aquatic forms (e.g. crustacea) may be expected to be highly susceptible to endocrine disrupters. We conclude that this field has been neglected rather than that effects do not exist. Many chemicals may also interfere indirectly, as is the case with for instance metals and apart from tributyltin, and we have not included these in the report.

THE CONTENT OF THE REPORT

Hormones are involved in regulation of several different and important processes in organisms. These include regulation of reproduction, development and metabolism. Chemical substances may disturb these functions by affecting hormonal signalling. In order to understand how hormonal systems are disturbed it is essential to have knowledge of their natural functions. The report has focused on three hormonal systems, the sex hormones (estrogens, androgens and progestins), thyroid hormones and retinoids. These are described in chapter 2.

Sex hormones regulate sex determination in many vertebrates, such as fish and reptiles. Exposure to chemical substances may therefore lead to changes in sex ratios in these species. The development of gonads and sexual behaviour is controlled by sex hormones in all vertebrates, including humans. The mechanisms for sex determination and differentiation are discussed in chapter 3.

A large number of different chemical substances are suspected to affect reproduction and development by interfering with hormones such as sex hormones, thyroid hormones and retinoids. Chapter 4 deals with demonstrated and discussed effects in humans and animals. A number of chemicals with potential hormone modulating effects are presented in chapter 5. The selected compounds are mainly those that have been reported to have endocrine disrupting capacity but a few compounds lacking such data have been included because of their presence in the environment and structural similarity to hormones or to other endocrine disrupting compounds. A multitude of experiments have been conducted to determine how different chemical substances interfere with hormones. Experimental studies of different endocrine mod- ulators are discussed in chapter 6. At the end of the report, in chapter 7, an evaluation of the observed and suspected effects are given together with recommendations for future research.

1. INTRODUCTION

The present report aims to give an up to date account of the literature on known and suspected disturbances of reproduction in vertebrates and to examine the possible association between observed effects, in both humans and wild-life, and chemicals that are released into the environment, with special emphasis on Swedish conditions. Following the evaluation of existing knowledge, recommendations for future research are given.

DEFINITION OF ENDOCRINE DISRUPTING SUBSTANCES (EDS)

It is recognized in this report that it is necessary to give a definition of endocrine disrupters. At the recent European workshop on the impact of endocrine disrupters on human health and wildlife, held in December 1996 in Weybridge, UK, (EUR 17549, 1996) it was agreed that *"An endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function"*. It was also agreed that *"a **potential** endocrine disrupter is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism"*. In this report we use these definitions. As these definitions suggest it is important to develop *in vivo* systems for determination of such chemicals while *in vitro* systems may be helpful in the selection of candidate substances. In the present report we have adopted the Weybridge definition of endocrine disrupters.

BACKGROUND

There are numerous incidences where humans or wildlife have been exposed to potential EDSs. In Taiwan, high levels of PCB and PCDF through contaminated oil resulted in several disorders in the children of exposed mothers (Rogan et al., 1988; Guo et al., 1993). In Florida, the alligators (*Alligator mississippiensis*) in Lake Apopka exhibit many reproductive and developmental abnormalities which have been suggested to be caused by EDSs (Clark 1990). In Western gulls (*Larus occidentalis*) in southern California skewed sex ratios with increased numbers of females and abnormal nesting behavior have been observed (Fox 1992). In fish outside paper mill and sewage treatment plants, skewed sex ratios, decreased testosterone levels and vitellogenin production in male fish have been reported from different parts of the world (Andersson et al., 1988; Munkittrick et al., 1991; Jobling and Sumpter 1993). In many parts of the world it has been observed that gastropods develop imposex (the development of a penis in females) when exposed to tributyltin from antifouling paint on ships (Bryan et al., 1986).

These observations have led to concern that environmental contaminants such as PCB, DDT, pentachlorophenol and alkylphenols may act as EDSs. Exposure to xeno- biotics with a potential to interfere with the normal hormone regulation may result in diminished fertility, altered sex differentiation, changes in behavior and altered cell differentiation leading to increased occurrence of cancer. Depending on the life stage when exposure occurs any or all of these effects may be induced. EDSs may thus induce changes in the normal development, cause different degrees of sex reversal and affect behavior.

Hormones are a diverse group of molecules that together with the nerve system coordinate different functions in the organism. The word hormone is derived from the Greek word *horman*, meaning to excite or stir up. Hormones are released by endocrine cells/organs and exert their action on other cells/organs after having been transported in the blood. Following synthesis in endocrine glands, hormones are released into the circulation and distributed throughout the body. While neural signaling substances act over short distances, generally moving a fraction of a micrometer across the synaptic cleft, hormones travel throughout the body in the circulation and may act far away from the site of release.

In the present report we consider the steroid hormones, the thyroid hormones and the retinoids. The steroid hormones are involved in the regulation of reproduction and the development of secondary sex characteristics. The thyroid hormones are involved in growth and differentiation and hypothyroidism leads to retarded growth and mental development. The retinoids are crucial for early embryonic differentiation and development and deficiency syndromes include effects on the reproductive systems. In addition to these hormones, many other signaling systems are involved during reproduction and developmental processes; and some of them act in concert with these hormones. Together these signaling systems form a central unifying regulatory system used by cells and tissues to integrate information relating to their state of differentiation and proliferation. Hormones may act locally and are sometimes multifunctional mediators of cellular events and their functions cannot be fully understood in isolation. Nevertheless, in the present evaluation we focus on the steroid, thyroid and retinoid signaling systems due to their proven critical roles in sex determination, reproduction and/or embryogenesis, and to demonstrated effects on these signaling systems by chemical pollutants in the environment.

The focus on EDSs has been on their interaction with the hormone receptors and the subsequent regulation of target genes. Following binding to the receptor, the chemicals may either stimulate or inhibit gene transcription in a manner similar to the natural hormone or they may inactivate gene transcription by forming receptor-ligand complexes with conformations that are unfavorable for activation. Some sub-

stances have, however, been found to exert both agonistic and antagonistic effects. Compounds having different mechanisms of action may cause similar biological changes. For instance, antagonists to the androgen receptor may give effects similar to those caused by estrogen receptor agonists. Besides interaction with hormone receptors, EDSs may interfere with transport proteins, alter the synthesis and biotransformation of hormones, have direct toxic effects on the gonads or have adverse effects on the hypothalamus, the pituitary or endocrine glands.

Historical background to the present research on persistent organic pollutants, causing reproductive disturbances, in Sweden

Persistent organic pollutants have long been indicated to be potential EDSs. Research on the occurrence and biological effects of persistent organic pollutants has a long tradition in Sweden. The Swedish pathologist Karl Borg suggested already in the 1950s that alkylmercury compounds could cause toxic effects in birds (Borg 1958). Seed-dressing with mercury salts had started around 1920 and alkylmercury was introduced as a dressing agent in the end of the 1940s. High mercury levels in dead white-tailed sea eagles (*Haliaeetus albicilla*) from the Baltic area were reported by Berg et al., (1966) and Henriksson et al., (1966). Westöö (1967) also identified methylmercury in bird eggs and other biological material. In the 1940s DDT was introduced on a large scale. In the late 60s ecologists at the Swedish Museum of Natural History started collecting material for monitoring of PCB and DDT levels. At this time, Jensen identified PCB as a contaminant in biota (Jensen, 1966) and high levels of DDT and PCB were subsequently found in various organisms from marine environments, especially in white-tailed sea eagles from the Baltic area (Jensen et al., 1969).

Synthesis of radioactively labelled and unlabelled individual chlorobiphenyls was initiated by Carl-Axel Wachtmeister at the Wallenberg Laboratory, Stockholm University (Sundström and Wachtmeister, 1973), which facilitated experimental studies of the toxicology of these compounds. By using the whole-body autoradiography technique developed by Ullberg (1954), tissue distribution of various chlorobiphenyls was studied by Berlin et al., (1975) and Brandt (1977). Following administration of certain labeled chlorobiphenyls to mice, it was found that radioactive substances accumulated in the respiratory and reproductive tracts. The compounds in the airways were identified as methylsulfonyl metabolites of the chlorobiphenyls (Bergman et al., 1979). The presence of methyl sulfone metabolites of DDE and PCB in environmental samples had earlier been reported by Jensen and Jansson (1976). The high concentrations of PCB and DDT observed in the Baltic at the beginning of the 1970s were suspected to be correlated to the observed decrease in reproductive capacity of the animals (Olsson et al., 1975). In order to study the reproductive toxicity of these compounds, investigations on mice (Örberg et al.,

1972; Kihlström et al., 1975) and mink (*Mustela* vison) were carried out (Jensen et al., 1977). A number of studies on PCB toxicity in mink have been performed in Sweden (Kihlström et al., 1992; Brunström et al., 1994; Bäcklin 1996).

In 1981, several 2,3,7,8-substituted polychlorinated dibenzo dioxins/furans (PCDD/Fs) were detected in grey seals (*Halichoerus grypus*) from the Baltic (Rappe et al., 1981). A broad survey of the dioxin contamination in Sweden was performed within a project called "The Swedish Dioxin Survey" which was administered by the Swedish Environmental Protection Agency and funded by the Swedish government. Experimental work on the toxicology of dioxins includes, e.g., studies of their teratogenic, immunotoxic, and tumor promoting effects (Hassoun et al., 1984, Dencker et al., 1985, Lundberg 1991, Flodström and Ahlborg 1989) as well as effects on retinoid levels and metabolism (Thunberg et al., 1979, Håkansson 1988, Hanberg 1996). Nordic risk assessments of dioxins and polychlorinated biphenyls have been carried out at the Institute of Environmental Medicine at the Karolinska Institute (Ahlborg et al., 1988; 1992). Most of the research on persistent organic pollutants in Sweden has been supported by the Swedish Environmental Protection Agency.

References

Ahlborg, U., Hanberg, A. and Kenne, K. (1992) Risk assessment of polychlorinated biphenyls (PCBs), Nord 1992:26.

Ahlborg, U., Håkansson, H., Wærn, F. and Hanberg, A. (1988) Nordisk dioxinriskbedömning. Nordiska Ministerådet, Köpenhamn, Miljörapport 1988:7. Nord 1988:49.

Andersson, T., Förlin, L., Härdig, J. and Larsson, Å. (1988) Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. 45:1525-1536.

Berg, W., Johnels, A. Sjöstrand, B. and Westermark, T. (1966) Mercury content in feathers of Swedish birds from the past 100 years. Oikos, 17:71-83.

Bergman, Å., Brandt, I. and Jansson, B. (1979) Accumulation of Methylsulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. Toxicol. Appl. Pharmacol., 48:213-220.

Berlin, M., Gage, J. and Holm, S. (1975) Distribution and metabolism of 2,4,5, 2',5'- pentachlorobiphenyl. Arch. Environ. Health, 30:141-147.

Borg, K. (1958) Inverkan av betat utsäde på viltfaunan. VIII Nord. Veterinärmöte, Helsingfors, 394-400.

Brandt, I. (1977) Tissue localization of polychlorinated biphenyls chemical structure related to pattern of distribution. Acta Pharmacol. Toxicol., 40:1-108.

Brunström, B., Bergman, A., Bäcklin, B.M., Lund, B.-O. and Örberg, J. (1994) Effects of long-term exposure to PCB and PCB methylsulfones on reproduction in the mink. Organohalogen Comp., 20:471-480.

Bryan, G.W., Gibbs, P.E., Hummersonte, L.G. and Burt, G.R. (1986) The dceline of the gastropod Nucella lapillus around south-west England: Evidence for the effect of tributyltin from antifouling paints. J. Mar. Biol. Ass. U.K. 66: 611-640.

Bäcklin, B-M. (1996) Studies on reproduction in femal mink (*Mustela vison*) exposed to polychlorinated biphenyls. Ph. D. Thesis, Swedish University of Agricultural Sciences, Uppsala.

Clark, D.R. (1990) Dicofol (Kelthane) as an environmental contaminant. In: U.S. Fish

and Wildlife Technical Report 29. Washington D.C. U.S. Fish and Wildlife Service. pp. 1-37.

Dencker, L., Hassoun, E., d'Argy, R. and Alm, G. (1985) Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its congeners. Mol. Pharmacol., 27:133-140.

EUR 17549 (1996) European workshop on the impact of endocrine disrupters on human health and wildlife. Report of Proceedings. pp 125.

Fox, G.A. (1992) Epidemiological and pathobiological evidence of contaminant-induced alterations in sexual development in free-living wildlife. In: Chemical-induced Alterations in Sexual and Functional Development: The wildlife/human conecction. Colborn, C.T. and Clement, C. (eds.) 23:147-158.

Flodström, S. and Ahlborg, U.G. (1989) Tumour promoting effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-effects of exposure duration, administration schedule and type of diet. Chemosphere, 19(1-6):779-783.

Guo, Y.L., Lai, T.J., Ju, S.H., Chen, Y.C. and Hsu, C.C. (1993) Sexual development in biological findings in Yucheng children. DIOXIN 93:13th International Symposium on Chlorinated Dioxins and Related Compounds, 14:235-238.

Hanberg, A. (1996)Effects of 2,3,7,8-tetrachlorodibenzo-r-dioxin on vitamin A storage in rat hepatic stellate cells. Ph. D. Thesis, Karolinska Institutet Stockholm.

Hassoun, E., d'Argy, R. and Dencker, L. (1984) Teratogenicity of 2,3,7,8-tetrachlorodibenzofuran in the mouse. J. Toxicol. Environ. Health, 14:337-351.

Henriksson, K., Karppanen, E. and Helminen, M. (1966) High residue levels of mercury in Finnish White-tailed eagles. Ornis Fennica, 43:38-45.

Håkansson, H. (1988) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the fate of vitamin A in rodents. Ph. D. Thesis, Karolinska Institutet, Stockholm.

Jensen, S. (1966) Report of a new chemical hazard. New Sci., 32:612.

Jensen, S. and Jansson, B. (1976) Anthropogenic substances in seal from the Baltic: Methyl sulfone metabolites of PCB and DDE. Ambio, 5:257-260.

Jensen, S., Johnels, A.G., Olsson, M. and Otterlind, G. (1969) DDT and PCB in marine animals from Swedish waters. Nature, 224:247-250.

Jensen, S., Kihlström, J.-E., Olsson, M., Lundberg, C. and Örberg, J. (1977) Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. Ambio, 6:239.

Jobling, S. and Sumpter, J.P. (1993) Detergent components in sewage effluent are weakly oestrogenic to fish: An *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquatic Toxicol. 27:361-372.

Kihlström, J-E., Lundberg, C., Örberg, J., Danielsson, P.O. and Sydhoff, J. (1975) Sexual functions of mice neonatally exposed to DDT or PCB. Environ. Physiol. Biochem., 5:54-57.

Kihlström, J-E., Olsson, M., Jensen, S., Johansson, Å., Ahlbom, J. and Bergman, Å. (1992) Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). Ambio, 21:563-569.

Lundberg, C. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of the thymus and on the specific immune response in the mouse. Ph. D. Thesis, Uppsala University.

Munkittrick, K.R., Port, C.B., Van der Kraak, G.J., Smith, I.R. and Rokosh, D.A. (1991) Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. Can. J. Fish. Aquat. Sci. 48:1371-1380.

Olsson, M., Johnels, A.G. and Vaz, R. (1975) DDT and PCB levels in seals from Swedish waters. The occurrence of aborted seal pups. Proceeding from the sympo-

sium on the seal in the Baltic. June 4-6, 1974, Lidingö, Sweden. SNV PM, 591:43-65.

Rappe, C., Buser, H.-R., Stalling, D.L., Smith, L.M. and Dougherty, R.C. (1981) Identification of polychlorinated dibenzofurans in environmental samples. Nature, 292:524-526.

Rogan, W.J., Gladen, B.C., Hung, K.L., Shih, L.Y., Taylor, J.S., Wu, Y.C., Yang, D., Ragan, N.B. and Hsu, C.C. (1988) Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334-336.

Sundström, G. and Wachtmeister, C.A. (1973) Synthesis of ^{14}C-labelled and unlabelled PCB compounds. PCB Conference II, National Swedish Environmental Protection Board, 4E:73-86.

Thunberg, T., Ahlborg, U.G. and Johansson, H. (1979) Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Arch. Toxicol., 42:265-274.

Ullberg, S. (1954) Studies on the distribution and fate of S^{35}-labelled benzylpenicillin in the body. Acta Radiol. (Stockolm), 118: 1-110.

Westöö, G. (1967) Determination of methylmercury compounds in foodstuffs. II. Determination of methylmercury in fish, egg, meat, and liver. Acta Chem. Scand., 21:1790-1800.

Örberg, J., Johansson, N., Kihlström, J.-E. and Lundberg, C. (1972) Administration of DDT and PCB prolongs oestrous cycle in mice. Ambio, 1:148-149.

2. HORMONE SYSTEMS

The genomic way of hormone action is to interact with nuclear receptors, which in turn bind to specific DNA-regions and activate or inhibit the transcription of specific mRNAs, leading to synthesis of specific proteins. Several intracellular hormone receptors have been isolated and characterized. They display a strong homology between each other and are regarded as a receptor-superfamily. It has been shown that several different isoforms exist of each of the receptors in the steroid hormone superfamily. The receptors activate genes through binding to a variety of different gene response elements.

Nuclear hormone receptors can be divided into steroid and non-steroid hormone receptors. The steroid hormone receptors, including the estrogen, androgen and progesterone receptors, bind to DNA as homodimers, whereas the non-steroid hormone receptors, including the receptors for thyroid hormones and retinoids, bind to their response elements either as homodimers or as heterodimers with the common partner, the retinoid X receptor (RXR). The genes for the receptors and binding proteins are highly conserved between species and show specific expressions in tissues and during development. The nuclear hormone receptors share a common structure and have a common mechanism of action. Receptors for many of these hormones have been cloned or identified from a number of different species (Table 2.1).

The nuclear hormone receptors in the steroid superfamily (except the glucocorticoid receptor) are located in the nucleus even in the absence of bound ligands. Steroid, thyroid and retinoid receptors have all been shown to regulate gene activity in the absence of hormone (Lees et al., 1989, Graupner et al., 1989, Tzukerman et al.,

TABLE 2.1. Nuclear receptors in various species. Note: C = receptor(s) cloned, B = receptors indicated to be present by binding studies.

ORGANISM	ESTROGEN	ANDROGEN	PROGESTIN	THYROID	RETINOID
Mammals	C	C	C	C	C
Birds	C	C	C	C	C
Reptiles	C	C	C	B	
Amphibians	C	C	B	C	C
Bony fish	C	B	C	C	C
Cartilaginous fish	B	B	B		
Cyclostomes	B				

1990, Baniahmad et al., 1990, Zhang et al., 1991a, Bamberger et al., 1996). This suggests that the receptors may have functions independent of their specific hormones.

In addition to the classical signaling pathway, recent research indicates that steroid hormones, thyroid hormones and retinoic acid regulate cellular events through non genomic pathways, such as membrane-receptor mediated signaling (Wehling 1994). The presence of membrane receptors has been deduced from functional experiments, often where hormones exert actions too rapid to be accounted for by the genomic mechanisms.

The activity of hormonal systems may thus be regulated at a variety of different levels, including the synthesis, transport, receptor interaction and excretion of the hormones. The metabolism and action of hormones is outlined in Table 2.2.

TABLE 2.2. Overview of hormone metabolism and action.

SYNTHESIS	Conversion from cholesterol to steroid hormone and from tyrosine to thyroid hormone. Retinoic acid is synthesized from dietary derived retinoids *in situ*.
TRANSPORT	Lipophilic hormones are transported bound to specific plasma transport proteins. For the hormones discussed these include the steroid hormone binding globulin (SHBG), transthyretin, thyroid binding globulin (TBG) and retinoid binding protein (RBP) In addition, dition, binding proteins transport retinol (CRBP) and retinoic acid (CRABP) in the cell.
CONVERSION	For the steroid hormones and the retinoids there are several active metabolites.
GENOMIC PATHWAYS	Activation through the nuclear receptors. Differences between tissues and organs exist. Slow effects.
NON-GENOMIC PATHWAYS	Activation through membrane receptors and other secondary pathways. Differences between tissues and organs exist. Rapid effects.
EXCRETION	Hydroxylation and or conjugation through phase I and phase II reactions leading to increased water solubility.

In conclusion, hormone systems may regulate gene expression and physiological functions by a multitude of different mechanisms. The presence of different pathways may account for the high variation in the responses to different exogenous compounds as will be discussed in Chapter 6.

Nuclear receptors

The best known mechanism by which hormones act is by binding to specific receptors. These hormone receptors comprise the nuclear hormone receptor family, which includes receptors for steroids, thyroid hormones, vitamin D3 and retinoids (Evans 1988).

The receptors can be divided into six regions (A-F) with regard to amino acid similarities in different parts of the protein (Krust et al., 1986). The most conserved region is the C region which is responsible for the DNA binding specificity. The ligand binding do- main is located in region E and is also con- served. The mechanisms of action are similar for these hormone receptors and below we use the estrogen receptor to exemplify the general mechanisms. A schematic representation of hormone receptors is given in Figure 2.1.

The main transcription-activating domain of these receptors is located in the ligand-binding domain (E-region) in the C-terminus (Webster et al., 1988). The receptors contain two inde- pendent acidic transcriptional activation function (AF) domains, AF-1 and AF-2 (Tora et al., 1989, Lees 1989). AF-1 is located in the N-terminal A/B-region and is responsible for constitutive activation, whereas AF-2 is hormone inducible and located within the E-region. The activities of AF-1 and AF-2 are cell type-dependent. Tora et al., (1989) proposed that these two activate different stages in initiation of transcription. In that way an interaction between different activating domains and different components of the basic initiation complex would be possible. It is not known how these domains stimulate transcription but it is probably through interactions with the transcription complex.

The receptors are inducible transcription factors that modulate specific gene expression by binding to short DNA sequences located on hormone regulated genes. These short DNA sequences are, in the case of estrogens, termed the estrogen response elements (ERE), and consist of inverted repeat sequences with the core sequence TGACCT (Parker 1990, Klein-Hitpass 1986, Klock et al., 1987). The consensus sequence of an ERE is 5'-AGGTCA nnnTGACCT-3'. Idealized EREs are organized as 2 half-sites and mediate estrogen response. The half-sites for hormone receptor binding have been determined and show that the receptors can be divided into two major groups. Glucocorticoids, aldosterone, progesterone and testosterone receptors all share the common DNA sequence TGTTCT, while estrogen, thyroid hormone, vitamin D3 and retinoid receptors all bind to TGACCT half-

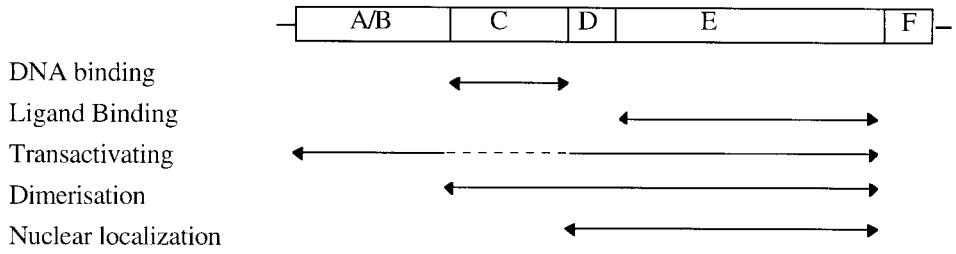


Figure 2.1 Schematic drawing of the organization of receptors in the nuclear hormone superfamily. Regions are defined as; A/B - N-terminal domain; C - DNA-binding; D - Hinge domain; E - Ligand binding and F - C-terminal domain.

sites (Umesono 1989). While the half-sites are shared between different hormone receptors, the spacing in-between the two half-sites may vary. It has been argued that the hormone itself interacts with DNA, thereby increasing the specificity of the DNA interaction (Hendry and Mahesh 1995).

The palindromic nature of the hormone response elements suggests that the receptors bind their targets as dimers (Kumar and Chambon 1988). A region within the hormone binding domain has been identifiedto be required for both hormone receptor dimerization and high affinity DNA-binding (Fawell et al., 1990). Experiments using point mutation of the murine ER has shown that there is a direct correlation between the ability of the different mutants to dimerize and their ability to bind to DNA (Fawell et al., 1990).

A recent hypothesis is that hormonal ligands in the steroid superfamily may act through insertion between base pairs in partially unwound DNA (Hendry and Mahesh 1995). This insertion hypothesis suggests that receptor bound ligands facilitate DNA unwinding, stereospecific control of donor/acceptor functional groups on the DNA followed by insertion and release of the ligand between base pairs at 5'-TG-3'-5'-CA-3'.

STEROID HORMONES

The steroid hormone family consists of different groups that are recognized by their physiological functions. In this report we are addressing three systems. They include the female secondary characteristic inducing hormones, estrogens (mainly 17β-estradiol, but also estrone and estriol); the male counterpart, androgens (mainly testosterone and its derivative 5α-dihydrotestosterone) and the progestins essential for pregnancy (progesterone, 17-hydroxyprogesterone and 17,20-dihydroxyprogesterone). In addition to regulating reproductive systems, the sex hormones also have effects on many other functions, such as growth, hemoglobin production and calcium metabolism in the skeleton.

Besides being synthesized in gonads, a number of steroid hormones are synthesized by the adrenal cortex. In *zona reticularis* glucocorticoids and small amounts of adrenal sex hormones are produced. The major part of the adrenal sex steroids are androgens but small amounts of estrogens and progesterone are also produced. Adrenal androgens are of little physiological importance in the male, but in adult women they are thought to play a role for the sex drive. When secreted in abnormal amounts, as in patients with congenital enzyme deficiencies in the adrenal gland or in patients with adrenal tumors, they have effects that depend on the sex and age of the individual. In prepubertal males and in females the effects can be dramatic. Females may develop a beard, a masculine pattern of body hair distribution and the clitoris may grow to resemble a small penis.

Estrogens

Estrogens have a central role in the control of both female and male reproduction. The estrogens are biosynthetically formed from androgens. They fulfill a range of roles in humans, some of which are only recently beginning to be understood. Estrogens do not only occur in females, but are also present in the male though generally at much lower levels. The naturally occurring estrogens are 17β-estradiol, estrone and estriol. These three are all C18-steroids.

Estrogen synthesis is regulated via the hypothalamo-hypophyseal-gonadal axis. Gonadotropin-releasing hormone is secreted from the hypothalamus and stimulates the pituitary to release gonadotropins into the blood, which subsequently stimulates the follicle cells in the ovaries to synthesize and secrete estrogens.

Once estrogen has entered the cell, it may bind to the estrogen receptor. Following binding, the receptor-ligand complex elicits its effect in the nucleus by upregulating the synthesis of different gene products. The expression of the estrogen receptor is tissue-specific as well as developmentally regulated (Kuiper et al., 1996). It is interesting to note that a novel prostate and ovary specific estrogen receptor (ER) has recently been cloned from rat (Kuiper et al., 1996). It is possible that the different estrogen receptors may have different ligand binding and transactivating properties. Furthermore, estradiol stimulates the synthesis of its own receptors (Mommsen and Lazier, 1986).

Recent studies of estrogen receptor mediated gene regulation have revealed that estrogenic responses are mediated by a multitude of different pathways and that the estrogen receptor may regulate gene activity in the absence of estrogen (Tzukerman et al., 1990, Sukovich et al., 1994).

Reproduction and development

Estrogens regulate the growth of the ovarian follicles and increase the motility of the uterine tubes. They also increase uterine blood flow and increase the smooth muscle mass of the uterus. Estrogens regulate the growth of the endometrium. It has been suggested that this is due to upregulation of the progesterone receptor (Bamberger et al., 1996). Estrogens produce duct growth in the breast and are important for estrous behavior in most mammals, apparently through acting directly on certain neurons in the hypothalamus. During puberty, estrogen is partially responsible for the development of breasts, uterus and vagina. Other effects of estrogen include induction of pigmentation of the areolas and retention of salt and water prior to menstruation.

Estrogen is necessary for the differentiation of female secondary sex characteristics. In the fetal brain amortization of androgens to estrogens is believed to be of importance for male sexual brain differentiation and later on for sexual behavior. The estrogen receptor is present already at the blastula stage and estrogen is pro-

duced at blastula implantation (Dickman and Day 1973, George and Wilson 1978, Hou and Gorski 1993). These studies indicate that both estrogen and estrogen receptors may be involved in early embryo development. It has been suggested that one effect of estrogen during early development may be regulation of Müllerian inhibitory hormone (MIH) and this is supported by the presence of a putative estrogen response element in the MIH gene promoter (Guerrier et al., 1990).

In mice, it has been shown that the intrauterine position during fetal life has an impact on the behavior later in life (vom Saal 1989a). Female mice which developed between two males, *in utero*, were found to have a more aggressive behavior (more tail-rattling) than females which had developed between two females (Palanza et al., 1995). The steroid hormone levels were also observed to be dependent on the positioning *in utero*. Thus, for instance, a male that developed between two males had higher testosterone levels than a male that developed between two females (vom Saal 1989a) and was also found to be more aggressive. Several other behavioral effects were also seen in these experiments, including effects on sexual activity, infanticide and reproductive capacity (vom Saal and Moyer 1985; vom Saal 1989a, vom Saal 1989b, Palanza et al., 1995). Since mouse embryos share a uterus, the steroids are able to pass between fetuses via diffusion through the amniotic fluid across the fetal membranes. The diffusion of steroids is believed to cause the changes in behavior observed in these mice, showing the importance of steroids in behavioral development.

Androgens

Testosterone is synthesized from cholesterol in the Leydig cells of the testis but also from androstenedione secreted by the adrenal cortex. All natural androgens are C19-steroids. The naturally occurring androgens are testosterone and 5α-dihydrotestosterone in mammals. In teleosts 11-ketotestosterone is also present and has been shown to stimulate development of male characteristics. Androgens increase protein synthesis and stimulate growth leading to increased growth rate.

Like estrogen, testosterone works primarily through interaction with nuclear receptors. The classic androgen receptors in mammals are intracellular and exert their effects via the genom (Bolander, 1989). The androgen receptor is regulated by endogenous levels of hormones. There are some conflicting data on the effect of androgens on androgen receptor mRNA levels (Jänne and Shan 1989; Abdelgadir et al., 1993; Gonzales-Cavadid et al., 1993). *In vivo* it appears that both testosterone and 5α-dihydrotestosterone down-regulate the androgen receptor mRNA levels. However *in vitro* it appears that testosterone down-regulates the androgen receptor while 5α-dihydrotestosterone upregulates it (Lin et al., 1993; Gonzales-Cavadid et al., 1993). The exact mechanism for androgen regulation of the androgen receptors still remains to be elucidated.

Reproduction and development

Androgens stimulate the appearance of masculine traits such as differentiation of male reproductive tracts, secondary male characteristics and spermatogenesis. 5α-dihydrotestosterone is more potent than testosterone in stimulating virilisation of the outer genitalia and growth of the prostate gland (Wilson 1992). The major conversion site of testosterone is the prostate gland where over 90% is converted to 5α-dihydrotestosterone by 5α-reductase. The production of 5α-dihydrotestosterone can be blocked with the aid of specific inhibitors (Mellin et al., 1993). Testosterone is responsible for male reproductive behavior and castration has been shown to diminish this behavior.

Production of testosterone during early development is necessary for the development of male genitals from the primordial genital ducts. Absence of testosterone leads to default development of female genitals. Testosterone is produced from the Leydig cells of the fetal testis while the Sertoli cells produce Müllerian inhibiting hormone. Both are necessary for development of male secondary sex characteristics. The development of male behavior is believed to occur in response to a peak in testosterone following birth. In rats it has been shown that exposure of females to androgens during the first days following birth results in male behavior.

Androgens in teleost fish

Teleosts utilize 11-ketotestosterone in addition to the mammalian type androgens. The hormone systems vary markedly between different teleost species. Testosterone is produced in the gonads of both sexes and displays increased plasma levels in prespawning and spawning fish. For a detailed account of the teleost hormone systems there are reviews available on gonadal steroids and androgens in teleosts (Fostier et al., 1983, Borg 1994). In most studied fish, including salmonids, the testosterone levels are higher in females than in males. However, the 11-oxygenated androgens, mainly 11-ketotestosterone occur at higher levels in males than in females. They are effective in stimulating male secondary sexual characteristics and at least in some fish, spermatogenesis and male reproductive behavior.

Receptors for testosterone have been found in some organs in fish, but the physiological function of testosterone is largely unknown. No traditional steroid receptors have been found for 11-ketotestosterone. As far as is known, 11-oxygenated androgens appear to be important hormones only in teleosts and sturgeons. In addition gonadal steroids and/or their derivatives have been shown to have a pheromonal function in many fish.

Progesterone

Progesterone, a C21-steroid, is formed from cholesterol and is converted from pregnenolone by 3β-hydroxysteroid dehydrogenase. It is an important intermediate in steroid hormone synthesis. Progesterone is secreted by the corpus luteum, placenta, the follicles and apparently also by the testes and adrenal cortex. The function of progesterone in males may only be as a biosynthetic intermediate. The hormone acts primarily on the uterus, breasts and brain.

There are two progesterone receptors and they have been shown to be generated from one single gene by alternative promoter usage (Kastner et al., 1990). The progesterone receptors are regulated by estrogen and possibly by glucocorticoids as well (Jeltsch et al., 1990). It has been shown that the B-isoform of the human progesterone receptor contains an N-terminal autonomous transactivation domain that is lacking in the A-isoform (Sartorius et al., 1994). This indicates that the two isoforms may perform different functions.

Reproduction

Progesterone secretion is increased by luteinizing hormone and prolactin. It is responsible for the progestational changes in the endometrium and the cyclic changes in mucus secretion in the cervix and vagina. Progesterone has antiestrogenic effects on the myometrial cells and also decreases the number of estrogen receptors in the endometrium. In breasts, progesterone stimulates the development of lobules and alveoli, induces differentiation of the ducts and maintains secretory functions during lactation.

Progesterone is involved in the regulation of endometrial proliferation and differentiation. Progestins have been shown to block estrogen induced uterine growth in rats (Kraus et al., 1993). Furthermore, the addition of antiprogestins, such as RU 486, prevent this progesterone mediated inhibition of uterine growth (Kraus et al., 1993). Estrogen has been shown to upregulate the progesterone receptor in the uterus (Manni et al., 1981) and both progesterone and estrogen have been shown to downregulate the uterine estrogen receptor (Manni et al., 1981, Hsueuh et al., 1976).

In a study using an endometrial adenocarcinoma cell line derived from epithelial cells it was shown that transfection with the progesterone receptor resulted in gene activation in the absence of progesterone (Bamberger et al., 1996). Addition of progesterone inhibited this activation. The estrogen receptor did not activate the studied genes, and estrogen did not block the progesterone receptor induced gene activity. The effect of progesterone receptors on endometrial proliferation in the absence of progesterone may thus be to regulate the hyperplasia of the endometrium during the early parts of the menstrual cycle. The increase in progesterone levels during the later part may explain the reversal of this effect.

PROGESTINS IN TELEOST FISH

In salmonid fish the main progestin is 17-hydroxy-20-dihydroprogesterone while other fish often have other types. These hormones occur in both sexes. In salmonids 17-hydroxy-20-dihydroprogesterone stimulates ovulation in females and spermiation (production of running milt) in males. 17-Hydroxy-20-dihydroprogesterone exerts its effects via membrane receptors.

Steroid hormone biochemistry

SYNTHESIS AND CATABOLISM

Steroid hormones are synthesized from cholesterol that, in turn, may be synthesized in steroidogenic tissues from acetate. Most of the cholesterol is however derived from low density lipoproteins or from high density lipoproteins. Free cholesterol is insoluble in the cytosol and is transported into the mitochondria by sterol carrier protein. Once it has reached the mitochondria, cholesterol can be cleaved into pregnenolone by cytochrome P450scc (side chain cleavage). This first conversion of cholesterol is a rate limiting step in steroid hormone synthesis. Following this step, pregnenolone can be modified to either progesterone or to 17OH-pregnenolone. Regardless of which of these pathways that is used, both cytochrome P450c17 and 3 -hydroxysteroid dehydrogenase are needed for the production of androstenedione. Testosterone is then synthesized from androstenedione. Finally a cytochrome P450 enzyme (aromatase) converts testosterone into estradiol. Aromatase is of particular interest since regulation of its activity influences the ratio of testosterone to estrogen which in turn is important for the development of sex specific traits in many vertebrates.

Finally the hormones need to be removed from the circulation and excreted. This is usually done by sulfate conjugation leading to inactivation and excretion via the urine. Sulfatation is thus an important pathway in the biotransformation of steroid and thyroid hormones. In humans there are several forms of sulfotransferase enzymes. These include the dehydroepiandrosterone sulfotransferase and two forms of phenol sulfotransferase. Dehydroepiandrosterone-sulfotransferase catalyses the sulfation of steroids such as dehydroepiandrosterone, estrone and estradiol.

CYTOCHROME P450 MEDIATED METABOLISM

Cytochrome P450s (CYP) are key enzymes in steroid synthesis but are also involved in the metabolism of other endogenous compounds, such as retinoids, thyroid hormones, fatty acids, prostaglandins, leukotrienes and biogenic amines. Many forms of CYP are also involved in the metabolism of foreign chemicals (xenobiotics) such as drugs, environmental pollutants and natural plant products. CYP comprises a multigene family of hemoproteins found in large abundance in the endoplasmatic reticulum of the liver, but can also be found in other tissues, although to a considerably lesser extent. CYP constitutes the monooxygenase (MO) system together with the membrane bound NAD(P)H-cytochrome c reductase, NAD (P)H-cytochrome b5 reductase and cytochrome b5. The oxidative biotransformations performed by the P450 MO system are also referred to as phase I reactions. These reactions are normally followed by conjugation reactions (also called phase II reactions) with a polar endogenous compound such as glutathione, glucuronic acid, sulfate or amino acids to facilitate excretion of the compounds.

A number of compounds are known to induce or inhibit the CYP system and/or the phase II enzyme reactions and can thus interact with the synthesis and biotransformation of hormones. Different compounds may however induce different forms of CYP and phase II conjugating enzymes. The inducible forms of CYP belong to the gene families CYP1 (polyaromatic hydrocarbon, PAH), CYP2 (phenobarbital/ethanol), CYP3 (steroid) or CYP4 (clofibrate). Examples of inducers of the different families are given in parenthesis.

Transport

Following secretion the steroid hormones bind to circulating plasma proteins. Since the steroid hormones are lipophilic they require protein binding in the plasma in order to be distributed throughout the body. The steroids bind specifically to two high affinity steroid-binding proteins, the sex-hormone-binding globulin and corticosteroidbinding globulin (Hammond 1993; Hammond and Bocchinfuso 1995), and nonspecifically to albumin. Be sides binding to the above mentioned transport proteins, progesterone also binds to corticosteroid-binding globulin. Testosterone has a higher affinity to sex-hormonebinding globulin than either estrogen or progesterone. The free, or unbound, fraction of the steroid hormones is considered to represent the biologically active fraction since it may enter target tissues/organs through diffusion from the capillaries.

Mechanism of action

DIRECT CONTROL OF GENE EXPRESSION

Steroid hormones regulate gene expression by interacting with specific intracellular receptors. These receptors are specific for the different hormones but may exist in multiple isoforms. Thus, there are at least two different isoforms of the estrogen receptor, the ERα and the ERβ, that are derived from different genes (Kuiper et al., 1996). The different isoforms of androgen and progesterone receptors have on the other hand

been shown to be different translation products from the same gene. The presence of different isoforms of steroid hormone receptors may be important for tissue specific functions.

Steroid hormones have been suggested to have a dual function in the mechanism by which the receptors activate transcription. As seen for estrogen, it may be an "unmasking" of the DNA-binding domain of the receptor since both ER-estrogen-complexes and ER-antiestrogen-complexes promote tight nuclear binding (Webster et al., 1988). Estrogen also appears to be required to activate a transcription-activating domain located within the hormone-binding domain (Webster et al., 1988). The antagonistic effects of the antiestrogens may be based on their inability to induce the transcription activation function of the ER (Webster et al., 1988).

Some substances have been found to exert both agonistic and antagonistic effects. This is most notable with many pharmaceutical chemicals such as tamoxifen and raloxifene.

It is believed that tamoxifen induces estrogenic responses through the N-terminal activation domain in the estrogen receptor, while it inhibits by blocking the C-terminal activation domain. The activation through the N-terminal domain is enough to give a weak estrogen response and tamoxifen has for instance been found to induce vitellogenesis in fish (White et al., 1994). However, when tamoxifen competes with estradiol for the binding to the receptor it down-regulates the vitellogenin response (White et al., 1994).

While tamoxifen is effective in treatment of hormone-dependent breast cancer, due to its estrogen antagonistic effects, the agonistic effects observed in the uterus indicate that tamoxifen treatment may not be without side effects. In a study of estrogen-induced calbindin-D gene expression Blin et al., (1995) observed that tamoxifen had both agonistic and partial anti-agonistic effects in ovariectomized rat uterus. Since tamoxifen is a weak estrogen-agonist in uterus there has been concern that tamoxifen treatment may promote uterine cancer.

Besides tamoxifen, raloxifene has also been shown to be an estrogen antagonist in breast tissue. However in contrast to tamoxifen, raloxifene is an antagonist in the uterus as well. Both substances function as agonists in bone tissue (Sato et al., 1996; Yang et al., 1996). There are thus distinct tissue specific patterns in the effects of estrogen, tamoxifen and raloxifene on breast, uterus and bone. Recent research has shed some light on the possible regulatory mechanisms behind these tissue specific effects of different pharmaceuticals and environmental endocrine disrupters.

The effects of estrogen in bone have been suggested to be independent of the classical genomic estrogen response elements (Yang et al., 1996). Instead of activating transcription through EREs, raloxifene confers its activity in bone through a new class of response elements, called raloxifene response elements (RRE) found in for instance the TGF-b3 gene. Yang and co-workers (1996) found that estrogen did not bind to RRE and activate TGF-b3 transcription. However, an intermediate metabolite of estrogen, 17-epiestriol did activate TGF-b3 in bone. This indicates that estrogen by-products may be the active forms in some tissues and that activation may be achieved through alternative genepromoter elements.

Since raloxifene is antagonistic in breast yet agonistic in uterus tissue there may be yet other mechanisms governing the tissue specificity of estrogens. It is therefore interesting to note that a novel prostate and ovary specific estrogen receptor (ERβ) has recently been cloned from the rat (Kuiper et al., 1996). Different estrogen receptors may have different ligand binding and transactivating properties. The presence of different pathways may account for the high variability in responses to different exoge-

nous compounds as will be discussed below.

The observed differences in tissue specific responses to estrogen antagonist and agonists may be dependent on which estrogen receptor that is being expressed, on which form of estrogen that is active and on which gene-promoter element that the receptor or the receptor-complex interacts with.

Another mechanism by which hormone receptors may be regulated is phosphorylation. Phosphorylation of the human ER by MAP kinase pathways may influence receptor action by a mechanism other than the estradiol-dependent phosphorylation of human ER by casein kinase II (Arnold et al., 1995.). Phosphorylation of the ER has been correlated with nuclear retention and specific DNA binding (Denton. et al., 1992). Steroid receptors may therefore not only regulate gene-expression following hormone-binding, but also in response to changes in the cellular milieu, through activated MAP kinase or AP-1.

It has been shown that the ER may bind to ERE and regulate gene activity in the absence of ligand (Lees et al., 1989, Tzukerman et al., 1990). Tzukerman and coworkers (1990) described two novel regulatory functions of the human ER, one constitutive activator function and one DNA binding function which enables the ER to bind and repress other activators of the ERE in the absence of estrogen. Thus, by binding to ERE in the absence of estrogen the receptor may function as a silencer of certain genes while allowing a constitutive basal activity of other genes.

Indirect control of gene expression

In some instances steroid hormones have been shown to exert effect on cells and tissues void of nuclear steroid hormone receptors. In many cases the responses in these regions have been too rapid to be attributed to transcriptional activation. These effects are believed to occur via membrane receptors. Thus, besides the classical activation pathway involving cytosolic receptors, steroids have also been indicated to exert effects at the cellular mem brane. Of special interest are the effects of estrogens and androgens on the development of the brain. Testosterone has been indicated in numerous studies to affect both behavior and neuroendocrine function of the brain. In the rat, it has been indicated that testosterone alters the pattern of estrogen receptor expression in the brain (Kühnemann et al., 1995).

Progesterone was reported to be active as a sedative already in 1941 by Seyle (1941). It has since been found that the inhibitory effect of steroids results from interaction with the gamma-aminobutyric acid-A ($GABA_A$) receptor, a class of ligand-gated Cl- channels, mediating inhibitory activity in the brain (reviewed by Paul and Purdy 1992; Sieghart 1992). In the hypothalamus, estradiol increases the turnover and release of GABA as well as the activity of GAD (glutamic acid decarboxylase) (Mansky et al., 1982; Duvilanski et al., 1983; Jarry et al., 1986).Thus, steroid hormones appear to be involved in GABAergic neuron activity by increasing the transcription of GAD mRNA thereby increasing the GABA synthesis, by increasing the presynaptic release of GABA and by prolonging the opening time of the post synaptic $GABA_A$ receptors. Taken together these effects indicate a dramatic effect of steroid hormones on the GABA activity. Recently a membrane progesterone has been identified in the plasma membrane of Atlantic croaker (*Micropogonias undulatus*) and spotted seatrout (*Cynosion nebulosus*) (Thomas et al., 1997). It has been indicated to be of importance for sperm motility in fish and recent evidence suggests that mammalian sperm also possess progeserone receptors (Thomas et al., 1997).

Several studies have now shown that steroids may bind to different membrane receptors including calcium channels, chloride

channels, muscarinic receptors and histamine receptors (Ben-Baruch et al., 1982; Brandes and Bogdanovic 1986; Hardy and Valverde 1994; Mermelstein et al., 1996).

NIH3T3 fibroblast cells, permanently transfected with MDR1 (multi drug resistant) cDNA, become insensitive to colchicine. When these NIH 3T3MDR cells were treated with estradiol, progesterone or tamoxifen in the presence of colchicine, it was found that estrogen inhibited chloride channels while antiestrogens activated them when the compound was added in the media (Hardy and Valverde 1994). This suggests that estrogens and antiestrogens may interact with the large conductance chloride channels.

17β-estradiol has been shown to reduce the calcium current in primary cultures of neostriatal neurones from rats (Mermelstein et al., 1996). Using whole-cell patch-clamp techniques 17β-estradiol was found to reversibly reduce Ba^{2+} entry through Ca^{2+} channels. The reduction of Ba^{2+} entry through Ca^{2+} channels is greater in female than in male rat neurones (Mermelstein et al., 1996). By conjugating 17β-estradiol to bovine serum albumin and thereby inhibiting cellular uptake, it was shown that the signals were mediated at the membrane surface. The hormonal effect on the calcium current was indicated to occur via G-protein activation (Mermelstein et al., 1996).

The above observations thus indicate that steroids have specific, rapid and non-genomic effects at the cellular membrane through interaction with binding sites distinct from the nuclear receptor.

THYROID HORMONES

The actions of thyroid hormones in higher organisms are critical for normal growth, differentiation and metabolic regulation (Legrand, 1986). Some of the most prominent effects of thyroid hormones occur during fetal development and early childhood. In humans, the requirements for thyroid hormones during development are shown dramatically in the syndrome of cretinism in which fetal hypothyroidism, often combined with maternal hypothyroidism, causes irreversible mental retardation and growth retardation if not treated early. Similarly, childhood hypothyroidism is characterized by striking impairment of linear growth. In adults, the primary effects of thyroid hormones are apparent by alterations in metabolism. Clinical features of hypothyroidism such as slowed mentation and speech, depression, hypothermia, skin changes, bradycardia, constipation, and reproductive dysfunction serve as reminders that thyroid hormones cause pleiotropic effects on many different organ systems.

The thyroid hormones are largely transported in the blood bound to thyroid binding globulin and transtyretin (Robbins et al., 1978). In adult rodents lacking thyroid binding globulin, more thyroid hormone is free of protein binding and therefore will be metabolized and excreted more easily. As a result, the half-life of thyroxine (T_4) in the rat is only about 12–24 hr in contrast to 6–7 days in humans. To compensate for increased turnover of thyroid hormone and to maintain physiological levels, the rat pituitary secretes more thyroid-stimulating hormone: As a comparison, the human baseline serum thyroid-stimulating hormone level is about 2.5 μU/ml, whereas in rats it ranges between 55 and 65 μU/ml in males and between 36 and 41 μU/ml in females. The resulting serum T_4 levels are 15–20 times higher in rats compared to humans.

The control of thyroid hormone synthesis and excretion is affected by a sensitive feedback mechanism that responds to changes in circulating levels of T_4 and triiodothyronine (T_3). The regulation system includes in addition to the thyroid gland also the hypothalamus and the anterior pituitary of the brain. An important peptide in this feedback system is the thyroid-stimulating hormone, which is secreted by the anterior pituitary gland and causes the thyroid to initiate new thyroid hormone synthesis and excretion. The effects of TSH on the thyroid appear to be the consequence of binding to cell-surface receptors and activation of adenyl cyclase and protein kinase with subsequent phosphorylation of cellular proteins. Cyclic adenosine monophosphate (cAMP) can mimic most of the actions of thyroid-stimulating hormone on thyroid cells. The rate of thyroid-stimulating hormone released from the pituitary is controlled by the amount of thyrotropin-releasing hormone secreted by the hypothalamus, and by the circulating levels of T_4 and T_3. If the levels of thyroid hormones are decreased, this will stimulate the secretion of thyroid-stimulating hormone leading to restoration of thyroid hormone levels; on the other hand, if exogenous thyroid hormone is administered, thyrotropin-releasing hormone secretion is suppressed and the thyroid will become inactive and eventually regress.

Studies on the regulation of thyroid-stimulating hormone output from the pituitary have indicated that a link exists between T_3 nuclear receptor occupancy and the mRNA levels for the thyroid-stimulating hormone subunit chains; administration of exogenous T_3 resulted in decreases in thyroid-stimulating hormone mRNA levels in the pituitary.

Furthermore, thyroid hormone responsive tissues contain a variable number of nuclear receptors for thyroid hormones (mainly T_3), usually several thousands per cell. Under euthyroid conditions in the rat, usually about 30–50% of the sites are occupied by T_3, although in the pituitary about 80% of the sites are filled. The T_3-receptor complex is quite labile with a half-life of ca 15 min.

Biochemistry

Synthesis

The thyroid hormones are synthesized in the thyroid gland and are stored as amino acid residues of thyroglobin, a complex glycoprotein constituting most of the colloid in the thyroid follicles. (The thyroid follicles consist of epithelial cells, thyrocytes, that surround a noncellular, proteinaceous substance, the colloid.) The first stage in the synthesis of the thyroid hormones is the uptake of iodide from the blood by the thyroid gland. The uptake requires energy and is effected by the so-called "iodide pump". Under normal conditions the thyroid may concentrate iodide up to about 50-fold its concentration in blood. Iodide uptake may be blocked by several anions (e.g. thiocyanate and perchlorate) and, since iodide uptake involves concurrent uptake of potassium, it can also be blocked by cardiac glycosides that inhibit potassium accumulation. In the next step, iodide is oxidized to an active iodine species that in turn iodinates the tyrosyl residues of thyroglobin. The reaction is catalyzed by a heme-containing peroxidase in the presence of hydrogen peroxide. The major products consist of diiodotyrosyl (DIT) residues, but monoiodotyrosyl (MIT) peptides are also formed. Additional reactions (which are thought to be catalyzed by the same peroxidase enzyme) involve the coupling of two DIT residues or of one DIT and one MIT residue, and lead to peptides containing residues of the two major thyroid hormones, i.e. thyroxine (T_4) and triiodothyronine (T_3).

The release of T_4 and T_3 from thyroglobulin is effected by endocytosis of colloid droplets into the follicular epithelial cells and subsequent action of lysosomal proteases. The free hormones are subsequently transported out from the epithelial cells into the circulation. Although T_4 is by far the major thyroid hormone excreted by the thyroid (normally 8 to 10 times the rate of T_3), it is usually considered to be a prohormone. Thus, T_3 is about fourfold more potent than T_4, and about 33% of the T_4 secreted undergoes 5'-deiodination to T_3 in the peripheral tissues; another 40% undergoes deiodination of the inner ring to yield an inactive material, reverse triiodothyronine (rT_3).

Transport

After entering the circulation, both T_4 and T_3 are transported bound, although not covalently, to plasma proteins (Robbins, 1991, De la Paz et al., 1992). In man, the major carrier protein is thyroxine-binding globulin (TBG), a glycoprotein that forms a 1:1 complex with the thyroid hormones. TBG has a high affinity for T_4 (K_a about 10^{10} M) and a lower affinity for T_3. This specific carrier protein is not present in rodents, cats and rabbits. Thyroxine-binding prealbumin, more often called transthyretin (TTR), and albumin also transport thyroid hormones in the blood; TTR has K_a values of about 10^7 and 10^6 M for T_4 and T_3, respectively. In the blood, TTR forms a complex with retinol-binding globulin, and this complex will therefore transport both thyroid hormones and retinol. In the normal situation, only ca 0.03% of the T_4 in the circulation is free and available for cell membrane penetration and thus hormone action, metabolism, or excretion. The levels of free thyroid hormones in the circulation may be changed through competitive binding interactions of certain drugs and other foreign compounds.

As indicated above, TTR is the major thyroid hormone transporter in rodents. However, during early stages of development TBG is expressed also in (at least some of) these animals (demonstrated in the euthyroid mouse). Between day 16 of fetal and day 60 of postnatal life, T_4 and T_3 binding in sera shows a striking ontogenic pattern, which largely could be explained by the presence of TBG: in fetuses, plasma protein

binding of thyroid hormones are 2–3 times higher than in mothers, and the binding further increases after birth, reaching between 3 and 5 days maximum values which are 7–8 times higher than the adult ones. It could be noted that the biosynthesis and/or secretion of TBG, but not of TTR, is under thyroid-hormone control, and experimental hypothyroidism will induce a marked increase in the serum TBG. The presence of TBG during early rodent developmental stages will make this model more attractive in extrapolating experimentally obtained thyroid hormone effects to man.

Metabolism and excretion

T_4, the major hormone secreted from the thyroid, is considered a prehormone and is converted to the more active T_3 by a 5'-monodeiodinase in a variety of peripheral tissues, including the pituitary. T_4 is also metabolized to rT_3 which is hormonally inactive and has no known function (except perhaps in feedback regulation). Under normal conditions the half-life of T_4 is 6 to 7 days in humans. Degradative metabolism of the thyroid hormones occurs primarily in the liver and involves conjugation with either glucuronic acid (mainly T_4) or sulfate (mainly T_3) at the phenolic hydroxyl group. The resulting conjugates are excreted in the bile into the intestine. A portion of the conjugated material is hydrolyzed in the intestine, and the resulting free hormones are reabsorbed into the blood. The remaining conjugated material is excreted in the feces.

Mechanism of action

Direct control of gene expression

The thyroid hormones act predominantly at the pretranslational levels selectively affecting gene expression, and this is achieved by the binding of T_3 to nuclear receptors closely associated to chromatin. The nuclear thyroid hormone receptors (TR) act as ligand-regulated transcription factors, which in turn act upon thyroid hormone response elements (TREs) in specific target genes. To some extent, thyroid hormone receptors act in conjunction with other hormone receptors (dimer formation) causing a modulation of the TRE-mediated binding to DNA. This suggests a possible complex regulation of gene expression with interactions ("cross-talk") between different hormone receptors and their response elements. The binding of T_3 to the TREs regulates transcription of adjacent target genes. Changes in gene transcription are reflected in alterations in mRNA levels and, subsequently, changes in protein biosynthesis. A number of genes that respond to thyroid hormone, either by being activated or repressed, have been identified.

What are the consequences of TR binding to TREs? In the absence of T_3, TRs generally have an inhibitory effect on basal gene transcription. Upon T_3 binding to the receptor, con formational changes will relieve basal repression and cause additional transcriptional enhancement. In this way, the TR acts as a potent transcriptional switch which can exert both repressive and stimulatory effects on transcription depending upon T_3 occupancy. The mechanism of induction is also suggested to involve other proteins which could modulate transcription (e.g. transcriptional adapter proteins).

Different forms of TRs have been isolated, TRα and TRβ. These two receptor isoforms are encoded by separate genes that are located on chromosomes 17 and 3, respectively. Both receptors bind thyroid hormones with high affinity, but differ in some properties. Through splicing additional isoforms would be created, and several subforms of both TR classes have been identified (e.g. TRα1 and α2; TRβ1 and β2). Notably, TRα2 no longer binds thyroid hormone, and therefore has been proposed to be a endogenous inhibitor of thyroid hormone receptor function. In general, the α and β receptor isoforms are distributed widely and

show overlapping patterns of expression. Spleen and testis are notable for their relative deficiencies of a1 and b1 receptors, a fact that could be correlated to data indicating that these tissues have minimal metabolic responses to thyroid hormone. TRβ2 is expressed predominantly in the pituitary and the hypothalamus. The α2 isoform is highly expressed in many tissues, particularly brain, kidney and testis.

Although TR can bind to selected DNA sequences as monomers, they generally bind with greater affinity as homodimers or as heterodimers with structurally related nuclear receptors. In addition to the thyroid hormone itself a variety of dimerization partners has been identified, and all are members of the nuclear receptor family. Examples are the RXRα, β and γ, i.e. retinoic acid receptor-related proteins. Other proteins (e.g. RAR) also dimerize with TR, although probably less well than RXRs. The binding of dimerization partners, such as RXR, will enhance receptor binding to DNA. We know that the tissue distribution of various RXR isoforms differs, but it is not clear whether there are differences in transcriptional enhancement of thyroid hormone receptor function by different partners. To conclude, ligands that bind to the dimerizations partners of TR have the potential of modulating transcriptional responses to TRs.

There is also suggested to exist a significant relationship between thyroid and estrogen hormones: a strong similarity exists between estrogen and thyroid hormone response elements (ERE, TRE) in target genes. It has been demonstrated that in the absence of TRs the TRE behaves similarly to imperfect EREs, which can synergize to mediate a strong estrogen-dependent activation of transcription. In the presence of TRs, however, the estrogen response from the TRE is strongly repressed.

Indirect control of gene expression

Thyroid hormones have been indicated to regulate cellular events through membrane-receptors (Wehling 1994). The presence of membrane receptors has been deduced from functional experiments, often where hormones exert actions too rapid to be accounted for by the genomic mechanisms.

RETINOIDS

Retinoids play a continuing role in many aspects of adult life, including normal growth and metabolism, vision, maintenance of numerous tissues, reproduction and overall survival (for review see Wolf 1980, Underwood 1984, Gudas et al., 1994). In addition, retinoids have a central role in differentiation and embryonic development (for review see Hofmann and Eichele 1994, Means and Gudas 1995).

Research during the last decade, starting with the discovery of retinoid specific receptors (Petkovich et al., 1987, Giguere et al., 1987) has dramatically changed the general perception of retinoids, both with regard to their physiological role and their mechanism of action. Beyond the classical action of retinol and its metabolites in nutrition and vision it has now become clear that retinoids are fundamental regulators of gene expression in all of the major vertebrate groups.

The major physiological forms of retinoids are retinol, retinal, retinoic acid and retinyl esters. Retinoic acid, which is the active form of the vitamin for most, if not all, of its functions, is the ligand for the nuclear retinoic acid receptor families, i.e. the retinoic acid receptors (RARα, β, χ) and the retinoid X receptors (RXRα, β, χ).

REPRODUCTION

Male sterility via a defect in spermatogenesis is a well recognized consequence of dietary vitamin A-deficiency in experimental animals (for review see Eskild and Hansson 1994). Degeneration and loss of germ cells take place, and a slight decrease in serum testosterone is observed (Appling and Chytil 1981, Huang et al., 1983, 1984). Retinol, but not retinoic acid, in the diet is able to restore the defect through a direct effect on the seminiferous epithelium (Catignani and Bieri 1980, Thompson et al., 1964). The inability of dietary retinoic acid to reinitiate spermatogenesis is likely to be a consequence of a blood-testis barrier for retinoic acid, and thus a requirement for its in situ synthesis (van Pelt et al., 1991). *In vitro* it has been demonstrated that retinol and retinoic acid stimulate the steroidogenesis in testicular cells (Chaudhary et al., 1989).

DEVELOPMENT

Inappropriate concentrations of retinoic acid during organogenesis are detrimental to normal embryogenesis. Excess or deficiency of vitamin A during this period both give rise to severe birth defects, including craniofacial malformations, abnormalities of the heart and thymus, skeletal malformations, reproductive and nervous system disorders (for review see Sucov and Evans 1995). The embryonic lesions occur only during defined windows in development and for any individual tissue this window is

very narrow, corresponding with the time at which that tissue undergoes differentiation. Studies in mice lacking RAR genes clearly demonstrate that RARs are essential for vertebrate ontogenesis and that retinoic acid is required at several stages of the development of numerous tissues and organs (Lohnes et al., 1994, Mendelsohn et al., 1994).

Retinoids are critically involved in the establishment of both the anterior/posterior central body axis, which forms the central nervous system and the spinal column, and later in the anterior/posterior specification of the limb, which forms a varied array of digits across this axis (for review see Means and Gudas 1995). It has been demonstrated that retinoid-specific binding proteins, enzyme activities and receptors are specifically expressed in the brain during development and it has also been suggested that retinoic acid is needed for neural crest cell survival, neurite outgrowth and hindbrain patterning (Maden et al., 1996). The specific expression of retinoic acid-generating aldehyde dehydrogenase activity in a subpopulation of dopaminergic neurons during development suggests a role for retinoic acid in non-electric information transmission of the central nervous system (McCaffery and Dräger 1994). Data from studies in the developing limb bud suggest that retinoic acid is involved in the patterning of cells along the anterior-posterior axis, as a signal between cells or tissues that can synthesize and secrete retinoic acid, and those that respond to this signal, e.g. by growth factor induction (Sucov and Evans 1995).

Observations in offspring of vitamin A-deficient rats (Wilson and Warkany 1948) and mice lacking the RAR genes (Mendelsohn et al., 1994) clearly demonstrate the retinoid requirement for proper morphogenesis of both the male and female reproductive tract. A pseudohermaphroditic tendency was observed in offspring of vitamin-A deficient rats. Male mice that lack the RARα gene are infertile, due to undeveloped testis and a defect in spermatogenesis (Lufkin et al., 1994). The ob served testicular changes resemble the ones observed in adult vitamin A-deficiency. Lack of the RXRβ gene also results in infertile male mice, which have a defect in spermatogenesis (Kastner et al., 1996). However, these changes are distinct from both RARα deficient mice and vitamin A-deficient animals. In the female, retinoids are necessary for normal epithelial differentiation of the uterus and for a full gestation, embryonic development and delivery (Thompson et al., 1964). Absence of the uterus and cranial vagina has been observed both in offspring of vitamin A-deficient rats (Wilson and Warkany 1948) and mice that lack the RAR genes (Mendelsohn et al., 1994). The observed female genital duct abnormalities, which in both cases were related to defects in the formation and midline union of the Müllerian ducts, suggest that retinoic acid is required for multiple steps in the morphogenesis of the female reproductive tract.

Biochemistry

Transport

Retinol bound to retinol-binding protein forms a complex with transthyretin in the blood. In contrast, there is no specific binding protein for the transport of retinoic acid in the plasma. Several intracellular retinoid-binding proteins, including the cellular retinol (CRBP and CRBPII) and retinoic acid (CRABP and CRABPII) binding proteins, exist and are believed to regulate the cellular levels of free retinol and retinoic acid, respectively, and to be involved in the retinoid metabolic pathways. In addition, the binding proteins serve to protect the ligands from degradation and to protect the cells from the membranolytic action of retinoids.

Synthesis and catabolism

In contrast to the classical hormones, which are synthesized, processed, and/or released in response to physiological cues, vitamin A (i.e. retinol) is a nutrient and most, if not all, organisms are dependent on its dietary intake, either as carotenoids from plants or as retinyl esters from animals. Under normal dietary conditions retinoids (i.e. retinol and retinyl esters) are present at relatively high concentrations and are therefore potentially available to all cells all the time. However, efficient biological systems, including retinoid-specific binding proteins, enzymes and nuclear receptors ensure proper uptake, storage, transport and conversion into the right concentration of active metabolites both on a temporal and local scale. Little is known about the role of the nuclear receptors in retinoid absorption and metabolism, whereas many aspects of the absorption and whole-body metabolism of retinoids have been well documented (for review, see Blaner and Olson 1994).

Briefly, dietary sources of vitamin A are absorbed and packaged as retinylesters of chylomicrons for secretion into lymph. Most of the newly absorbed chylomicron retinyl esters are cleared from plasma and are taken up by liver parenchymal cells (hepatocytes). After hydrolysis and reesterification, retinyl esters are stored in lipid droplets in both hepatocytes and liver perisinusoidal stellate cells. Stored retinyl esters are mobilized and delivered to target tissues as retinol bound to retinol-binding protein (RBP) in serum. The signal triggering the release of stored retinyl esters is not known. In target tissues, retinol is converted into storage forms, active metabolites, or catabolic products. Lecitin and acyl coenzyme A: retinol acyl transferases (LRAT; ARAT) and retinylester hydrolases (REH) are the two enzyme systems involved in retinoid storage processes. Retinoid-specific alcohol and aldehyde dehydrogenases catalyze the reversible conversion of retinol into retinal, and the irreversible conversion of retinal into retinoic acid, respectively. Both retinol and retinoic acid undergo reversible and irreversible interconversions to more polar metabolites, some of which are functional, and others which are inactive catabolic products. The cytochrome P450 system is involved in these reactions and intense research is ongoing to define retinoid-specific cytochrome P450 isozymes and reactions in various tissues. These enzymes may be very important in the regulation of the level of free retinoic acid. Retinol and retinoic acid as well as its oxidized metabolites are conjugated with glucuronic acid both *in vivo* and *in vitro*. The reaction is facilitated by microsomal uridine diphosphoglucuronosyl transferases.

Mechanism of action

Direct control of gene expression

Intense research in the retinoid field during the last decade has demonstrated an enormous complexity in retinoid-signaling at the molecular level (Chambon 1994, Mangelsdorf et al., 1994). It is now known that effects of retinoids are mediated via two families of nuclear retinoic acid receptors each composed of three subtypes, i.e., RARα, β, χ and RXRα, β, χ (Mangelsdorf et al., 1994 for review). Multiple forms of each of the receptor subtypes, receptor ligands (all-trans retinoic acid and 9-cis retinoic acid), response elements (RAREs), and intracellular binding proteins (CRBP and CRABP) add to the complexity. Furthermore, in order to bind to DNA, RARs and several other members of the nuclear receptor family require heterodimerization with RXR. Thus, by acting through RXRs, retinoids are critically involved in several different hormonal signaling pathways, including the thyroid hormones and vitamin D3.

There is also evidence that retinoids act on estrogen signaling pathways. Studies *in vitro* show that retinoic acid, via receptors, interferes with estrogenic action (Demirpence et al., 1994). Inhibition of the estrogen-response can occur either via inhibited binding of the estrogen receptor to the estrogen responsive element or via downregulation of target genes, containing retinoic acid responsive elements, in the estrogen receptor-pathways. In MCF7-cells, it is known that retinoic acid regulates the expression of both the estrogen receptor and downstream estrogen-induced genes (Rubin et al., 1994). It was, e.g., demonstrated that retinoic acid regulates the expression and reductive activity of the 17β HSD-activity, which converts estrone into estradiol (Reed et al., 1994). The 17β HSD gene contains a putative RARE sequence. Estrogen responsive genes may also be negatively regulated by RXRb through two distinct inhibitory pathways (Segars et al., 1993). Furthermore, there are a number of retinoic acid-responsive genes that are likely to be involved in developmental processes (for review see Gudas et al., 1994). These include transcription factors (e.g. Hox genes and N-myc), cell adhesion molecules (e.g. laminin B1), growth factors (e.g. PDGF, TGFβ and BMPs) and growth factor receptors (e.g. EGF receptor and PDGF receptor).The patterns of expression of the various RAR and RXR isoforms indicate that most tissues are potential targets of retinoid action (Giguere 1994).

Indirect control of gene expression

RARs can regulate gene transcription without binding to specific DNA-sequences, but by virtue of highly specific protein-protein interactions with other transcription factors (Yang Yen et al., 1991, Zhang et al., 1991b). Cross-coupling appears to be one mechanism by which RARs, in response to their hormone, can regulate the function of other transcription factors such as the protooncogene complex AP-1 through both protein-protein and DNA binding interactions (Mangelsdorf et al., 1994).

References

Abdelgadir, S.E., Connolly, P.B. and Resko, J.A. (1993) Androgen regulation of androgen receptor messenger ribonucleic acid differs in rat prostate and selected brain areas. Mol. Cell. Neurosci. 4:532-537.

Appling, D.R., Chytil, F. (1981) Evidence of a role for retinoic acid (vitamin A-acid) in the maintenance of testosterone production in male rats. Endocrinology 108: 2120-2123.

Arnold, S. F., Obourn, J.D., Jaffe, H. and Notides, A.C. (1995) Phosphorylation of the human estrogen receptor by mitogen-

activated protein kinase and casein kinase II: Consequence on DNA binding. J. Steroid Biochem. Molec. Biol. 55: 163-172.

Bamberger, A.-M., Bamberger, C.M., Gellersen, B. and Schulte, H.M. (1996) Modulation of AP-I activity by the human progesterone receptor in endometrial adenocarcinoma cells. Proc. Natl. Acad. Sci. USA 93:6169-6174.

Baniahmad, A., Steiner, C., Kohne, A.C. and Renkawitz, R. (1990) Modular structure of a chicken lysozyme silencer: involvement of an unusual thyroid hormone receptor binding site. Cell 61:505-514.

Ben-Baruch, G., Schreiber, G. and Sokolovsky, M. (1982) Cooperativity pattern in the interaction of the antiestrogen drug clomiphene with the muscarine receptors. Mol. Pharmacol. 21:287-293.

Blaner, W.S. and Olson, J.A. (1994) Retinol and retinoic acid metabolism. Retinoids: Biology, Chemistry, and Medicine, Second Edition 229-255.

Blin, C., L'Horset, F., Leclerc, T., Lambert, M., Colnot, S., Thomasset, M. and Perret, C. (1995) Contrasting effects of tamoxifen and ICI 182 780 on estrogen-induced calbindin-D 9K gene expression in the uterus and in primary culture myometrial cells. J. Steroid. Biochem. Mol. Biol. 55:1-7.

Bolander, F. (1989) Kintetics. In "Molecular endocrinology". Bolander, F. (ed.) Academic Press Inc. London. 3:63-76.

Borg, B. (1994) Androgens in teleost fishes. Comp. Biochem. Physiol. 109C: 219-245.

Brandes, L.J. and Bogdanovic, R.P. (1986) New evidence that antiestrogen binding site may be a novel growth promoting histamine receptor. Biochem. Biophys. Res. Commun. 126:905-910.

Catignani, G.L. and Bieri J.G. (1980) Ineffectiveness of testosterone and FSH in maintaining the vitamin A deficient rat testis. Nutrition and Metabolism 24:255--260.

Chambon, P. (1994) The retinoid signaling pathway: molecular and genetic analyses. Cell Biol 5:115-125.

Chaudhary, L.R., Hutson, J.C. and Stocco, D.M. (1989) Effect of retinol and retinoic acid on testosterone production by rat Leydig cells in primary culture. Biochemical and Biophysical Research Communications 158:400-406.

De la Paz, P., Burridge, J.M., Oatley, S.J. and Blake, C.C.F. (1992) Multiple modes of binding of thryoid hormones and other iodothyronines to human plasma transthyretin. In: The design of drugs to macromolecular targets (ed. C.R. Bedell) John Wiley & Sons Ltd, N.Y. 119-171.

Demirpence, E., Balaguer, P., Trousse, F., Nicolas, J.C., Pons, M. and Gagne, D. (1994) Antiestrogenic effects of all-trans-retinoic acid and 1,25- dihydroxy-vitamin D3 in breast cancer cells occur at the estrogen response element level but through different molecular mechanisms. Cancer Res 54:1458-1464.

Denton, R.R., Koszewski, N.J. and Notides, A.C. (1992) Estrogen receptor phosphorylation. Hormonal dependence and consequence on specific DNA binding. J. Biol. Chem. 267:7263-7268.

Dickman, Z., and Day, S.K. (1973) Two theories: the preimplantation embryo is a source of steroid hormones controlling (1) morula-blastocyst transformation and (2) implantation. J. Reprod. Fertil. 35: 625-617.

Duvilanski, B., Munoz-Maines, V. and Debeljuk, L. (1983) GABA-related enzymes in the hypothalamus of rats treated with estradiol. Eur. J. Pharmacol. 89: 259-264.

Eskild, W., Hansson, V. (1994) Vitamin A functions in the reproductive organs. Marcel Dekker Inc. New York.

Evans, R.M. (1988) The steroid and thyroid hormone receptor superfamily. Science 240:889-895.

Fawell, S.E., Lees, J.A., White, R. and Parker, M.G. (1990) Characterization and colocalization of steroid binding and dimerization activities in the mouse estrogen receptor. Cell. 60:953-962.

Fostier, A., Jalabert, B., Billard, B and Zohar, Y. (1983) The gonadal steroids. In "Fish Physiology". Hoar, W.S., Randall, D.J. and Donaldson, E.M. (eds.) Academic Press, New York. vol 9:277-372.

George, F.W. and Wilson, J.D. (1978) Estrogen formation in the early rabbit embryo. Science 199:200-201.

Giguère, V. (1994) Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. Endocrine Reviews 15:61-79.

Giguère, V., Ong, E.S., Segui, P., Evans, R.M. (1987) Identification of a receptor for the morphogen retinoic acid. Nature 330:624-629.

Gonzales-Cavadid, N.F., Vernet, D., Fuentes Navarro, A., Rodriguez, J.A. Swerdloff, R.S. and Rafjer, J. (1993) Up-regulation of the levels of androgen receptor and its mRNA by androgens in smooth-muscle cells from rat penis. MOl. Cell. Endocrinol. 90:219-229.

Graupner, G., Wills, K.N., Tzukerman, M., Zhang, X.K., Pfahl, M. (1989) Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity. Nature 340:653-656.

Gudas, L.J., Sporn, M.B., Roberts, A.B. (1994) Cellular Biology and Biochemistry of the Retinoids. Retinoids: Biology, Chemistry, and Medicine, Second Edition 443-520.

Guerrier, D., Boussin, L., Mader, S., Josso, N., Kahn, A. and Picard, J.Y. (1990) Expression of the gene for anti-Mullerian hormone. J. Reprod. Fretil. 88:695-706.

Hammond, G.L. and Bocchinfuso, W.P. (1995) Sex hormone-binding globulin/ androgen-binding protein: steroid-binding and dimerization domains. J-Steroid-Biochem-Mol-Biol. 53:543-52.

Hammond, G.L. (1993) Extracellular steroid-binding proteins. Steroid hormone action. Parker, M.G. (ed.). Oxford University Press Inc. New York. U.S.A. 1:1-17.

Hardy, S.P. and Valverde, M.A. (1994) Novel plasma membrane action of estrogen and antiestrogens revealed by their regulation of a large conductance chloride channel. FASEB J. 8:760-765.

Hendry, L.B. and Mahesh, V.B. (1995) A putative step in steroid hormone action involves insertion of steroid ligands into DNA facilitated by receptor proteins. J. Steroid Biochem. Molec. Biol. 55:173-183.

Hofmann, C. and Eichele, G. (1994) Retinoids in Development. Retinoids: Biology, Chemistry, and Medicine, Second Edition 387-441.

Hou, Q. and Gorski, J. (1993) Estrogen receptor and progesterone receptor genes are expressed differentially in mouse embryos during preimplantation. Proc. Natl. Acad. Sci. USA 90:9460-9464.

Hsueuh, A.J., Peck, E.J. and Clark, J.H. (1976) Control of uterine estrogen receptor levels by progesterone. Endocrinol. 98:438-444.

Huang, H.F.S. and Marshall, G.R. (1983) Failure of spermatid release under various vitamin A states – An indication of delayed spermiation. Biol Reprod 28: 1163-1172.

Huang, H.F.S., Zaidi, P. and Nieschlag, E. (1984) Pituitary-testicular interrelationships during germinal involution in the vitamin A deficient rat. Journal of Endocrinology 100:33-41.

Jänne, O.A. and Shan, L. (1989) Structure and function of the androgen receptor. Ann. New York Acad. Sci. 626:81-91.

Jarry, H., Sprenger, M. and Wuttke, W. (1986) Rates of release of GABA and cathecolamines in the medial basal hypothalamus of ovariectomized and ovariectomized estrogen-treated rats: correlation with blood plasma prolactin levels. Neuroendocrinol. 44:422-428.

Jeltsch, J.-M., Turcotte, B., Garnier, J.-M., Lerouge, T., Krozowski, Z., Gronemeyer, H. and Chambon, P. (1990) Characterization of multiple mRNAs originating from the chicken progesterone receptor gene. J. Biol. Chem. 265: 3967-3974.

Kastner, P., Bocquel, M.T., Turcotte, B., Garnier, J.M., Horwitz, K.B. Chambon, P. and Gronemeyer, H. (1990) Transient expression of human and chicken progesterone receptors does not support alternative translational initiation from a single mRNA as the mechanism generating two receptor isoforms. J. Biol. Chem. 265: 12163-12167.

Kastner, P., Mark, M., Leid, M., Gansmuller, A., Chin, W., Grondona, J.M., Decimo, D., Krezel, W., Dierich, A., Chambon, P. (1996) Abnormal spermatogenesis in RXR mutant mice. Genes and Development 10:80-92.

Klein-Hitpass, L., Schorpp, M., Wagner, U. and Ryffel, G.U. (1986) An estrogen-responsive element derived from the 5´flanking region of the xenopus vitellogenin A2 gene functions in transfected human cells. Cell 46:1053-1061.

Klock, G., Strahle, U. and Schutz, G. (1987) Oestrogen and glucocorticoid responsive eements are closely related but distinct. Nature 329:734-736.

Kraus, W.L. and Katzenellenbogen, B.S. (1993) Regulation of progesterone receptor gene expression and growth in the rat uterus: Modulation of estrogen actions by progesterone and sex steroid hormone antagonsits. Endocrinol. 132: 2371-2379.

Krust, A., Green, S., Argos, P., Kumar, V., Walter, P., Bornert, J.M. and Chambon, P. (1986) The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors. EMBO J. 5:891-897.

Kuhnemann, S., Brown, T.J., Hochberg, R.B. and MacLusky, N.J. (1995) Sexual differentiation of estrogen receptor concentrations in the rat brain: effects of neonatal testosterone exposure. Brain. Res. 691:229-234.

Kuiper, G.G.J.M., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson J.-Å. (1996) Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA 93:5925-5930.

Kumar, V. and Chambon, P. (1988) The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. Cell 55:145-156.

Lees, J.A., Fawell, S.E. and Parker, M.G. 1989. Identification of constitutive and steroid-dependent transactivation domains in the mouse oestrogen receptor. J-Steroid-Biochem. 34:33-9.

Legrand, J. (1986) Thyroid hormone effects on growth and development. In: Thyroid hormone metabolism (ed. Hennemann, G.), Marcel Dekker Inc. N.Y. 503-534.

Lin, M.C., Rajfer, J., Swerdloff, R.S. and Gonzales-Cavadid, N.F. (1993) Testosterone down-regulates the levels of androgen receptor mRNA in smooth muscle cells from rat corpora cavernosa via aromatization to estrogens. J. Steroid. Biochem. Mol. Biol. 45:333-343.

Lohnes, D., Mark, M., Mendelsohn, C., Dollé, P., Dierich, A., Gorry, P., Gansmuller, A. and Chambon, P. (1994) Function of the retinoic acid receptors (RARs) during development. (I) Craniofacial and skeletal abnormalities in RAR double mutants. Development 120: 2723-2748.

Lufkin, T., Lohnes, D., Mark, M., Dierich, A., Gorry, P., Gaub, M.P., Lemeur, M., Chambon, P. (1993) High postnatal lethality and testis degeneration in retinoic acid receptor alpha Mutant mice. Proc Natl Acad Sci USA 90:7225-7229.

Maden, M., Gale, E., Kostetskii, I., Zile, M. (1996) Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. Current Biology 6:417-426.

Mangelsdorf, D.J., Umesono, K., Evans, R.M. (1994) The retinoid receptors. Retinoids: Biology, Chemistry, and Medicine, Second Edition 319-349.

Manni, A., Baker, R., Arafah, B.M. and Pearson, O.H. (1981) Uterine osestrogen and progesterone receptors in the ovariectomized rat. J. Endocrinol. 91:281-287.

Mansky, T., Mestres-Ventura, P. and Wuttke, W. (1982) Involvement of GABA in the feedback action of estradiol on gonadotrpoin and prolactin release: hypothalamic GABA and cathecolamine turnover rates. Brain Res. 231:353-364.

McCaffery, P., Dräger, U.C. (1994) High levels of a retinoic acid-generating dehydrogenase in the meso-telencephalic dopamine system. Proc Natl Acad Sci USA 91:7772-7776.

Means, A.L., Gudas, L.J. (1995) The roles of retinoids in vertebrate development. Annual Review of Biochemistry 64: 201-233.

Mellin, T.N., Busch, R.D. and Rasmusson, G.H. (1993) Azasteroids as inhibitors of testosterone 5 alpha-reductase in mammalian skin. J. Steroid. Biochem. Mol. Biol. 44:121-131.

Mendelsohn, C., Lohnes, D., Decimo, D., Lufkin, T., Lemeur, M., Chambon, P., Mark, M. (1994) Function of the retinoic acid receptors (RARs) during development. 2. Multiple abnormalities at various stages of organogenesis in RAR double mutants. Development 120: 2749-2771.

Mermelstein, P.G., Becker, J.B. and Surmeier D.J. (1996) Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. J. Neurosci. 16: 595-604.

Mommsen, T.P. and Lazier, C.B. (1986) Stimulation of estrogen receptor accumulation by estradiol in primary cultures of salmon hepatocytes. FEBS Lett. 195:269-271.

Palanzia, P., Parmigiani, S. and vom Saal, F.S. (1995) Urine marking and maternal aggression of wild female mice in relation to anogenital distance at birth. Physiol. Behav. 58:827-835.

Parker, M.G (1990). Mechanisms of action of steroid receptors in the regulation of gene transcription. J. Reprod. Fert. 88: 717-720.

Paul, S.M. and Purdy, R.H. (1992) Neuroctive steroids. FASEB J. 6:2311-2322.

Petkovich, M., Brand, N.J., Krust, A. and Chambon, P. (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330: 444-450.

Reed, M.J., Rea, D., Duncan, L.J. and Parker, M.G. (1994) Regulation of estradiol 17-hydroxysteroid dehydrogenase expression and activity by retinoic acid in T47D breast cancer cells. Endocrinology 135:4-9.

Robbins, J. (1991) Thyroid hormone transport proteins and the physiology of hormone binding. In: Werner and Ingbar's The Thyroid: a fundamental and clinical text. -6th ed. (ed. Braveman L.E. and Utiger R.D.) J.B. Lippincott co. Philiadelphia. 111-125.

Robbins, J., Cheng, S., Gershengorn, M.C., Glinoer, D., Cahnmann, H.J. and Edelnoch, H. (1978) Thyroxine transport proteins of plasma. Molecular properties and biosynthesis. Rec. Prog. Horm. Res. 34:477-517.

Sartorious, C.A., Melville, M.Y., Hovland, A.R., Tung, L., Takimoto, G.S. and

Horwitz, K.B. (1994) A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segement of the B-isoform. Mol. Endocrinol. 8:1347-1360.

Sato, M., Rippy, M.K. and Bryant, H.U. (1996) Raloxifene, tamoxifen, nafoxidine. or estrogen effects on reproductive and nonreproductive tissues in ovariecomized rats. FASEB J. 10:905-912.

Segars, J.H., Marks, M.S., Hirschfeld, S., Driggers, P.H., Martinez, E., Grippo, J.F., Wahli, W. and Ozato K (1993) Inhibition of Estrogen-Responsive Gene Activation by the Retinoid-X Receptor-beta – Evidence for Multiple Inhibitory Pathways. Mol Cell Biol 13:2258-2268.

Seyle, H. (1941) The anesthetic effect of steroid hormones. Proc Soc. Exp. Biol. Med. 46:116-121.

Sieghart, W. (1992) Molecular basis of pharmacological heterogeneity of $GABA_A$ receptors. Cell Signal. 4:231-237.

Sucov, H.M. and Evans, R.M. (1995) Retinoic acid and retinoic acid receptors in development. Mol Neurobiol 10: 169-184.

Sukovich, D.A., Mukherjee, R. and Benfield, P.A. (1994) A novel, cell-type-specific mechanism for estrogen receptor-mediated gene activation in the absence of an estrogen-response element. Mol. Cell. Biol. 14:7134-7143.

Thomas, P., Breckenridge-Miller, D. and Detweiler, C. (1997) The teleost membrane receptor: A novel site of action of xenoestrogens. Proceedings of the Ninth International Meeting on Pollutant Responses in Marine Organisms, Bergen, Norway. p 182.

Thompson, J.N., Howell, J.M. and Pitt, G.A.J. (1964) Vitamin A and reproduction in rats. Proc Roy Soc B 159:-510-535.

Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E. and Chambon, P. (1989) The Human Estrogen Receptor Has Two Indepenent Nonacidic Transcriptional Activation Functions. Cell 59:477-487.

Tzukerman, M., Zhang, X.K., Hermann, T., Wills, K.N., Graupner, G. and Pfahl M. (1990) The human estrogen receptor has transcriptional activator and repressor function in the absence of ligand. New. Biol. 2:613-620.

Underwood, B.A. (1984) Sporn, M.B., Roberts, A.B. and Goodman, D.S. (eds) The Retinoids Volume 1. Academic Press, Inc. Orlando, USA, pp 281-392.

Umesono, K. and Evans, R.M. (1989). Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell. 57:1139-1146.

van Pelt, A.M.M. and de Rooij, D.G. (1991) Retinoic acid is able to reiniate spermatogenesis in vitamin A-deficientrats and high, replicate doses support the full development of spermatogenic cells. Endocrinology 128:697-704.

vom Saal, F.S. (1989a) Sexual differentiation in litter-bearing mammals: influence of sex on adjacent fetuses in utero. J. Anim. Sci. 67:1824-1840.

vom Saal, F.S. (1989b) The production of and sensitivity to cues that delay puberty and prolong subsequent oestros cycles in female mice are influenced by prior intrauterine position. J. Reprod. Fertil. 86: 457-471.

vom Saal, F.S. and Moyer, C.L. (1985) Prenatal effects on reproductive capacity during aging in female mice. Biol. Reprod. 32:1116-1126.

Webster, N.J.G., Green, S., Jin-Jia, R. and Chambon P. (1988). The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function Cell 54:199-207.

Wehling, M. (1994) Novel aldosterone

receptors: specificity-conferring mechanism at the level of the cell membrane. Steroids 59:160-163.
Wilson, J.D. (1992) Syndromes of androgen resistance. Biol. Reprod. 46:168-173.
Wilson, J.G. and Warkany, J. (1948) Malformations in the genito-urinary tract induced by maternal vitamin A deficiency in the rat. Am. J. Anat. 83:357-407.
White, R., Jobling, S., Hoare, S.A., Sumpter, J.P. and Parker, M.G. (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinol. 135: 175-182.
Wolf, G. (1980) Vitamin A. In: Alfin-Slater RB, Kritchevsky D. (eds) Human nutrition, a comprehensive treatise. Plenum Press, New York, pp 97-203.
Yang, N.N., Venugopalan M., Hardikar S. and Glasebrook A. (1996) Identification of an estrogen response element activated by metabolites of 17b-estradiol and raloxifene. Science 273:1222-1225.
Yang-Yen, H.F., Zhang, X., Graupner, G., Tzukerman, M., Sakamoto, B., Karin, M. and Pfahl, M. (1991) Antagonism between retinoic acid receptors and AP-1: implications for tumor promotion and inflammation. New Biologist 12: 1206-1219.
Zhang, X.K., Wills, K.N., Graupner, G., Tzukerman, M. , Hermann, T. and Pfahl, M. (1991a) Ligand-binding domain of thyroid homrone receptors modulate DNA binding and determines their bifunctional roles. New Biol. 3:169-181.
Zhang, L.X., Cooney, R.V. and Bertram, J.S. (1991b) Carotenoids Enhance Gap Junctional Communication and Inhibit Lipid Peroxidation in C3H/10T1/2 Cells – Relationship to Their Cancer Chemopreventive Action. Carcinogenesis 12: 2109-2114.

3. SEX DETERMINATION AND DIFFERENTIATION

GONADAL SEX DETERMINATION

Vertebrates are generally gonochoristic, i.e. each specimen is either a male or a female. Intersexuality and hermaphroditism are rare except among the teleost fishes. Sex determination in a strict sense is the process by which the gonad-anlage differentiates into either a testis or an ovary. This is not to be confused with the further development and maturation of the gonads. Except among the teleost fishes, the testis is formed from the medulla of the gonad anlage and the ovary from the cortex. Subsequently, the gonads control the development of most other characters that are different between the sexes via their hormone production. Vertebrate sex determination can be either genetical (as in all mammals and birds) or controlled by environmental factors, i.e. temperature (as in many poikilothermic vertebrates). Relevant for a discussion on possible effects of endocrine disrupters is not which genetic and/or environmental factors are involved but rather whether the process can be influenced by hormones.

TABLE 3.1 Determination of gonadal sex in vertebrates.
Note: XY = male heterogamety, ZW = female heterogamety, Temp. = temperature influences sex determination, Androgen Masculin. = androgens may induce male gonadal sex, Estrogen Femin. = estrogens may induce female gonadal sex. X refers to that the phenomenon occurs within the group, it is not necessarily widespread. (X) refers to that the phenomenon has only been found in a few cases and/or to a limited extent. In reptiles and amphibians both temperature and genetic factors may be involved in the same species. In amphibians and bony fish both XY and ZW systems may occur in closely related species, or in a frog, even within the same species.

	XY	ZW	Temperature	Androgen Masculin.	Estrogen Femin.
Mammals	X	–	–	–	(X)*
Birds	–	X	–	(X)	X
Reptiles	X	X	X	(X)	X
Amphibians	X	X	X	(X)	X
Bony fish	X	X	X	X**	X**
Cartilaginous fish		Information lacking			
Cyclostomes		Information lacking			

*) only in marsupials
**) occurs both in fish with XY and ZW sex determination

Mammals

In mammals, the male is the heterogametic sex, producing gametes with different sex chromosomes, X and Y. Presence of the Y chromosome leads to masculinization of the gonads. The gonadal sex determination in eutherian mammals is not mediated by steroids and is thus not likely to be influenced by endocrine disrupters.

Birds

In birds, the female sex is the heterogametic (ZW) and the female phenotype actively differentiates in response to estrogen. Sex differentiation in the chicken begins in the 7-day-old embryo, when increasing amounts of estrogens are produced by the ovaries (Weniger & Zeis, 1971, Weniger, 1974). In female birds, morphological sex differentiation is asymmetrical and only the left gonad differentiates into an ovary whereas the right ovary remains rudimentary. Several studies have demonstrated the importance of estrogens in bird sex determination by sex reversal of genetic males after estrogen treatment or of genetic females after treatment with aromatase inhibitors that prevent the formation of estrogens (Elbrecht and Smith, 1992).

Reptiles

The sex of most snakes and lizards is determined by sex chromosomes and both male and female heterogamety occurs. However, in all crocodilians, many turtles, and some lizards, the temperature of the developing eggs is the determining factor. Three different patterns occur: 1) low temperatures give rise to males, high to females, 2) low temperatures give rise to females, high to males, 3) intermediate temperatures give rise to males, high and low to females (for review, see Dournon et al., 1990). Even a small difference in temperature can produce drastically different sex ratios. As in genetic sex determination, there is a critical period in which the sex can be irreversibly determined. This period occurs in mid-embryonic development. Intersexuality is rare in reptiles and the sex, once determined, does not change. Gonadal sex depends on the temperature activation of genes encoding for steroidogenic enzymes and hormone receptors. Similarly as in birds, many studies have shown estrogens to stimulate differentiation in the female direction (Crews, 1996).

Amphibians

Amphibians have been less studied in this respect than the amniotes, but there are known cases of both male and female heterogamety as well as temperature dependent determination. In some species sex determination can be influenced both by temperature and by genetic factors and can be very different also between closely related species, as in Pleurodeles (Dournon et al., 1990). Estrogens may change genetic

males into phenotypic females and androgens have been observed to have the opposite effect in the frog *Buergeria buergeria* (Ohta 1987).

Bony fish

Most bony fish are gonochoristic, but functional hermaphroditism also occurs in many species, which is extremely rare in other vertebrates. Most hermaphrodites are sequential, i.e. they start being a male (protandry) or a female (protogyny) and later change into the other sex. Sex changes are accompanied by changes in steroid hormone patterns, but little is known about the extent to which changes in steroid patterns control the sex change rather than the other way around. There are fish with male as well as with female heterogamety. In addition, there are species in which environmental factors, such as temperature at early ontogenetic stages will decide into which sex the fish will differentiate (Strussmann and Patino, 1995). The sex determination in fish can be influenced by steroid hormones even in gonochoristic species (for review, see Hunter and Donaldson 1983, Borg 1994). There can be a reversal in the female direction with estrogens and, in addition, there are numerous studies showing drastic masculinizing effects by early androgen treatment, which has only been found in few cases or to a limited extent in other classes of vertebrates. The androgens used have mostly been testosterone or its synthetic derivatives, but 11-oxygenated androgens (which are the most important androgens in teleosts, see below) will also give these effects. If the dose of androgens used is very high a paradoxical feminization may occur, i.e. the proportion of males will start to decrease. This effect is found using aromatizable androgens (that can be converted to estrogens) but not using non-aromatizable androgens (Piferrer and Donaldson 1991).

GENITAL DUCTS

During the early embryonic stages the fetus of tetrapods, primitive bony fishes and of cartilaginous fish has primordia for both female and male genital ducts, the Müllerian and Wolffian ducts respectively. This system does not occur in teleosts and cyclostomes. The embryonic mammalian testis secretes testosterone which induces the differentiation of the Wolffian duct system into epididymis, vas deferens and seminal vesicles. In the absence of testosterone this system largely disappears. The mammalian and avian testes also produces a protein hormone, Müllerian inhibitory hormone which causes the disappearance of the Müllerian ducts, which would otherwise have differentiated into oviduct and uterus. The general pattern in birds and reptiles appears to be similar as in mammals, although far less is known. Only the left Müllerian tube differentiates in female birds, as does only the left ovary.

Estrogens also stimulate the Müllerian tubes in birds and reptiles, whereas physiological levels of estrogens have little effect in mammals.

OTHER SEXUAL DIFFERENCES

The further differentiation of sexual ducts, sexual glands, genitalia, secondary sexual characters gamete formation and reproductive behavior is in most studied cases dependent on steroid hormones (androgens in males, estrogens or estrogens and progestins in females) in vertebrates of all classes. The effects of testosterone are in many cases dependent on conversion of testosterone into other steroids. Reduction of testosterone to 5α-dihydrotestosterone is of critical importance for the differentiation of many secondary sexual characteristics and parts of the male reproductive tracts in tetrapods. Aromatization of testosterone to estradiol is of critical importance for differentiation of the brain in a male direction and for the control of male sexual behavior in birds and mammals.

REFERENCES

Borg, B. (1994) Androgens in fishes. Comp. Biochem. Physiol. 109C: 219-245.

Crews, D. (1996) Temperature-dependent sex determination: The interplay of steroid hormones and temperature. Zool. Sci. 13:1-13.

Dournon, C., Houillon C. and Pieau C. (1990) Temperature sex reveral in amphibians and reptiles. Int. J. Dev. Biol. 34: 81-92.

Elbrecht, A. and Smith, R.G. (1992) Aromatase activity and sex determination in chickens. Science 255:467-470.

Hunter, C.S. and Donaldson, E.M. (1983) Hormonal control and its application to fish culture. In W.S. Hoar, D. Randall, E.M. Donaldson eds. Fish Physiology, 9B, Academic Press, New York, pp. 223-303.

Ohta, S. (1987) Sex determining mechanism in Buergeria buergeria (Schlegel). 2. The effects of sex hormones on the differentiation of gonads and the offspring of sex-reversed females. Sci. Rep. Lab. Amphibian Biol. Hiroshima Univ. 9: 213-238.

Piferrer, F. and Donaldson, E.M. (1991) Dosage-dependent differences in the effect of aromatizable and non-aromatizable androgens on the resulting phenotype of coho salmon (*Oncorhynchus kisutch*). Fish Physiol. Biochem. 9:145-150.

Strüssmann, C.A. and Patiño, R. (1995) Temperature manipulation of sex differentiation in fish. In F.W. Goetz, P. Thomas eds. Proceedings of the fifth international symposium on the reproductive physiology of fish. Fish Symp 95, Houston. pp. 153-157.

Weniger, J.P. (1974) Sur l'induction de la synthese d'estrogenes par les androgenes. C.R. Acad. Sci. Hebd. Seances. Acad. Sci. D. 279: 145-151.

Weniger, J.P. and Zeis, A. (1971) Biosynthese d'oestrogenes par les ebauches gonadiques de poulet. Gen. Comp. Endocrinol. 16, 391-395.

4. DEMONSTRATED OR DISCUSSE EFFECTS IN HUMANS AND ANIMALS

HUMAN STUDIES

There are several effects on humans that have been suggested to be linked to environmental exposure to endocrine disrupting chemicals. These include developmental effects, reduced sperm quality and increased cancer incidence.

In Taiwan people were exposed to high levels of PCBs and PCDD/Fs through contaminated rice oil (Rogan et al., 1988). Children born to mothers who had consumed the contaminated oil showed pre- and postnatal growth retardation, hyper- pigmentation and chloracne, widely open fontanels, spotty calcification of the skull, abnormalities in natal teeth and small head circumference, elevated incidence of bronchitis, neurodevelopmental delays (including hypoactivity and hypotony) lower mean intelligence quotients, increased auditory thresholds, abnormal sexual organ development, reduced birth weight, neurological disorders, and at puberty the boys had significantly shorter penises than a control group (Rogan et al., 1988, Chen et al., 1994, Guo et al., 1993).

Behavioral effects

Several human prospective epidemiological studies have indicated that PCBs may affect the development of brain functions (Rogan et al., 1986, Rogan et al., 1988, Jacobson et al., 1990, Guo et al., 1993, Jacobson and Jacobson 1996). In Taiwan and Japan, neurological disorders have been observed in children born to mothers who had ingested high levels of PCB in cooking oil (Guo et al., 1993). In the Lake Michigan area it has been indicated that children of mothers who consume large quantities of fish high in PCBs showed signs of developmental disturbance including poorer short term memory (Jacobson et al., 1990). In another study, from North Carolina, gross motor function disturbances were observed in children as a result of exposure to PCB and DDE (Rogan et al., 1986). In a recent epidemiological follow up study, effects on intellectual functions were correlated to high levels of PCB exposure (Jacobson and Jacobson 1996). Impairments were observed in general intellectual ability, short and long term memory, focused and sustained attention in children with PCB levels exceeding 1.25 μg/g blood fat. The effects were suggested to be primarily due to transplacental exposure. This period has also been shown to be vulnerable to PCB exposure in experiments on rats (Lilienthal and Winneke 1991). Support for a transient effect of PCB/dioxins on the early behavior of infants was indicated in a

series of Dutch studies on infants born in a semi-urban (Groeningen) and a highly industrial area (Rotterdam). It was indicated that a combination of high intrauterine and high postnatal exposure to PCB during the first two weeks of breast feeding may result in neurological non-optimality at birth (Huisman et al., 1995). Moreover, transient negative effects on mental and psychomotor development during the first months of life was associated with high intrauterine PCB/dioxin exposure. At the age of 18 months, however, no strong association between PCB/dioxin exposure and negative developmental effects was found (Huisman et al., 1995; Koopman-Esseboom et al., 1996). Epidemiological studies of Swedish fishermen showed an increased risk for low birthweight for infants among mothers with a consumption of fatty fish from the Baltic Sea (Rylander et al.,1995, 1996) and for those mothers with the highest concentrations of PCB in plasma (Rylander et al., 1997). The plasma levels of PCBs in fishermen's wives from the Swedish east coast correspond well to the exposure levels for the Dutch mothers from whom the exposure levels of PCB were correlated to an increased risk for delayed neurodevelopment in infants (Grimvall et al., 1997, Koopman-Esseboom et al., 1994).

Testicular and prostate cancer

Several forms of hormone related cancers, including testicular cancer and prostate cancer in men, and breast cancer in women, have been indicated to be increasing in the Nordic countries. The possible involvement of environmental endocrine disrupters in hormone related cancer is not known. However, alterations in hormonal responses or in the metabolism of hormones have not been ruled out as a cause of hormone related cancers.

Testicular cancer mostly affects men in their late twenties and is one of the most common cancer forms in this age group. Testicular cancer has increased dramatically during the last 40 years. In Sweden the incidence has increased from about 2 per 10,000 in the 1950s to 4 per 10,000 in the late 1980s. Over the same time period the incidence has increased from 4 to 9 in Denmark and from 3 to 7 in Norway. Even in Finland, with its low frequency of testicular cancer, there has been a noticeable increase from 1 to 2 incident cases per 10,000 men (Adami et al., 1994). It has been proposed that testicular cancer is initiated already before birth. Changes in life-style and diet have been suggested to play an important role and especially the diet of the mother may be of importance. In Finland, the incidence of testicular cancer is three times higher in big cities than in rural areas (Suominen and Vierula, 1993).

Prostate cancer is the most common form of cancer in men in Sweden. The incidence rate of prostate cancer per 10,000 men is now 5.5 in Sweden, 5 in Norway, 3.5 in Finland and 3 in Denmark, and it has approximately doubled during the last 35 years. The prostate is stimulated by androgens, and castration and anti-androgen ad-

ministration are standard methods in the treatment of prostate cancer. However, androgens can also be aromatized to estrogens in the prostate and the estrogens act together with androgens in stimulating prostate growth (Suzuki et al., 1996).

Breast and uterine cancer

Breast cancer is the most common form of cancer in women in Sweden. It is clearly associated with estrogen and one of the most important treatments is by administration of the estrogen antagonist tamoxifen. The possibility that environmental estrogens may cause breast cancer has been reviewed by Davis et al., (1993) and Ahlborg et al., (1995) and only a brief summary will be given here: Breast cancer has been increasing in the industrialized world since 1940. This increase cannot be fully explained by known risk factors, such as low age of menarche, high age of menopause, late pregnancy, and dietary factors in general. It has been suggested that environmental estrogens may be involved in the increasing incidence of breast cancer. The rationale has been that many chlorinated organic compounds have estrogenic effects, that some organochlorines have been found to promote mammary carcinogenesis in animal experiments, and that some studies (but not others) have demonstrated higher levels of several organochlorines in breast tissue or blood in patients with breast cancer than in controls. However, the hypothesis that exposure to hormonally active chemicals would promote human breast cancer remains to be verified.

The increased incidence of endometrial cancer has been indicated to be influenced by exogenous estrogen treatment (reviewed in Cohen and Rahaman 1995 and Ahlborg et al., 1995). Endometrial proliferation and differentiation is under the influence of estrogen and progesterone. It has been suspected for a long time that overproduction of estrogen may lead to increased growth of the endometrium (Gusberg 1947). Estrogen replacement therapy has been reported to result in a drastically increased risk of developing endometrial cancer (Antunes et al., 1979, Rubin et al., 1990). Treatment of patients with breast cancer with Tamoxifen has been indicated to increase the incidence of endometrial cancer (Seoud et al., 1993). The risk of endometrial cancer is reduced by addition of progestins to patients treated with Tamoxifen (Fornander et al.,1989). Treatment with estrogen agonists such as Tamoxifen and exposure to estrogenic substances from the environment may thus induce permanent activation of the target genes, leading to endometrial hyperplasia and possibly tumor formation.

Hypospadias

A male reproductive tract malformation that has been reported to be increasing in many Western countries is hypospadia (WHO 1991). In Sweden there have been reports of about 20 cases per 10,000 births since the mid 1970s, compared to below

15 in the late 1960s. There have also been reports of increased numbers of registered cases of hypospadias over a 20 year period (1970 to 1990) in Norway (from 5–10 to 15–20 cases per 10,000) and Denmark (from about 7 cases to about 12 cases per 10,000). In Finland there are fewer cases, (5 per 10,000 births). Hypospadias has also been reported as increasing in England and Wales, from about 14 cases per 10,000 in 1965 to 36 per 10,000 in 1983. However, the reporting of hypospadias may not have been properly validated and its correlation to EDSs has not been established.

Treatment of pregnant women with the synthetic estrogen, diethylstilbestrol (DES) has been shown to lead to increased incidence of hypospadias in the sons of these women (see Jensen et al., 1995). This suggests that exogenous estrogens may have the potential to induce this malformation.

Cryptorchidism

The incidence of cryptorchidism, i.e. testes that do not descend properly, has been reported to be increasing. When comparing the results from two British studies, one performed in the late 1950s (Scorer 1964) and the other during the 1980s (Ansell et al., 1992), it appears that the incidence of cryptorchidism increased from 1.74% to 5.2% in 3-month-old boys with a birth weight under 2500 g, and from 0.91% to 1.61% in boys with a birth weight over 2500 g. No correlation to EDSs has been established for the occurrence of cryptorchidism. However, when pregnant women were treated with DES it was observed to lead to increased incidence of cryptorchidism in the sons of these women (see Jensen et al., 1995). This indicates that exogenous estrogens have the potential to induce this malformation.

Sperm quality

It has been proposed that sperm quality has declined and that this could be due to EDSs (Carlsen et al., 1992; Skakkebaek and Keiding 1994). A decline in semen quality would certainly be serious. However, two points are controversial. **1)** Has there been a general decline in semen quality? **2)** If so, is this due to EDSs?

1) Carlsen et al., (1992) noted a general drop in human sperm counts, and a decrease in ejaculate volumes, in a survey of 61 studies on sperm counts conducted between 1938 and 1991 in several countries worldwide. The study has, however, been much criticized: the different studies from which the data have been compiled were not performed similarly, there were differences in selection of subjects, sampling procedure and evaluation (Olsen et al., 1995). There was also a geographical bias (Fisch and Goluboff 1996) in the data compiled by Carlsen et al., (1992) since all large ($n \geq 100$) studies older than 1971 were performed in the United States, and all but one from New York, where sperm counts (Fisch et al., 1996) are especially

high. There are also studies showing declining trends within different countries, e.g. by Bostofte et al., (1983) in Denmark and Bendvold (1989) in Norway. However, no declining trend was observed in Finland between 1958 and 1992, where counts were high compared with other studies (Suominen and Vierula, 1993). In another study sperm count was correlated to the year of birth of the donor (Irvine 1994). In donors born in 1940 the sperm count was 120 million per ml whereas those born in 1949 had 75 million sperm per ml. Comparisons between fertility studies based on time showed a small but significantly higher degree of fertility in Finland than in Britain (Joffe 1996), though there were some minor methodological differences. The difference in fertility might, however, be due to factors other than sperm quality.

Decreasing sperm quality has, however, also been found in studies using similar methods throughout. Auger et al., (1995) observed a decrease in sperm counts from 89 to 60 million per ml, in percentage of motile and morphologically normal sperm, but not in sperm volume between 1973 and 1992 in Parisian sperm donors, in a carefully controlled study. Comhaire et al., (1995) observed a decrease in sperm concentration in sperm donors in Belgium over the period 1977–1994. There was, however, an increase in semen volume over the same period so the total sperm count did not change. There was also a decline in sperm motility. Ginsburg et al. (1994) observed a decline in sperm counts from 1978–1983 to 1984–1989 in the Thames Water Supply area, but not in other parts of the UK.

However, in other studies no decline in sperm quality was observed. Paulsen et al., (1996) found no decrease in sperm concentration, semen volume, number of sperm per ejaculate and percent normal sperm morphology in healthy men in the Seattle area between 1972 and 1993. On the contrary there was a small but statistically significant increase in sperm concentration. Similarly, Fisch et al., (1996) found no decline in sperm counts, semen volume or sperm motility in semen collected in Minnesota, New York and California between 1970 and 199., On the contrary, in the former two areas there was a significant increase in sperm counts. The sperm counts reported from New York were similar to those reported from this area in the early 1950s and earlier.

In conclusion, a decline in sperm quality has occurred in some areas, but probably not in others. There is no Swedish investigation, and the trends from the most comparable countries are highly different.

2) Sharpe and Skakkebaek (1993) suggested that declining sperm counts and rising incidences in male reproductive disturbances could be due to rising exposure to estrogen. This suggestion was based on the observation that these disturbances are similar to those observed in men exposed to the synthetic estrogen diethylstilbestrol *in utero* and to those observed in rats exposed during early ontogeny to small doses of estrogen. It can also be suspected that estrogenic exposure has increased, not only due to EDSs, but also to an increased consumption of foods containing estrogens

(dairy products and soy beans), slower clearance of estrogens due to a decreased intake of dietary fibers and increased estrogen production in fat due to increased obesity (Sharpe and Skakkebaek 1993).

Other mammalian studies

Florida panther

The Florida panther (*Felis concolor*) exhibits many reproductive and developmental abnormalities and these have been suggested to be caused by endocrine disrupting chemicals (Facemire et al., 1995). The male specimens showed low sperm concentration and ejaculation volume, as well as poor sperm motility and a high incidence of abnormal sperm. Cryptorchidism has been reported to be increasing among the panthers. Mercury poisoning has been suggested to be the cause of observed thyroid dysfuntions. The panthers have high tissue levels of many xenobiotics, including PCB. It has, however, also been suggested that the poor reproductive status of Florida panthers may be due to inbreeding, since the population consists of only 30–50 animals (O'Brien et al., 1994) and the genetic variation in the animals is low (Barone et al., 1994). Although the reproductive disturbances seen in Florida panthers may be due to inbreeding, it is too early to dismiss EDSs as a contributing factor.

Seals and sea lions

In the 1960s the numbers of seals in the Baltic decreased dramatically and they disappeared from many parts of the Baltic coast. Analysis of DDT and PCB in the beginning of the 70s showed that tissues of Baltic seals contained high concentrations of these contaminants, and this was suggested to be connected to a high incidence of aborted seal pups in the southern parts of the Baltic (Olsson et al., 1975). Similarly, abortions and premature births in Californian sea lions (*Zalophus californianus*) were suspected to be caused by organochlorine contamination (Delong et al., 1973). Among ringed seals (*Phoca hispida*) and grey seals (*Halichoerus grypus*) in the Baltic, a high prevalence of uterine occlusions was found in the mid 70s (Helle et al., 1976). Various pathological changes in Baltic seals have been reported (Bergman and Olsson 1986). The disease complex was suggested to be due to hyperadrenocorticism caused by a primary lesion of the adrenals followed by secondary changes in other organs. Between 1950 and 1975 the population of common seals (*Phoca vitulina*) in the western part of Wadden Sea, The Netherlands declined from more than 3000 to less than 500 animals. PCBs were thought to be responsible for the low rate of reproduction in the seals. When common seals were fed a diet consisting of fish from the western part of the Wadden Sea, their reproductive success was re-

duced (Reijnders, 1986). Several experimental studies in mink have shown that PCB may impair reproduction when the residue levels in the animals are similar to those found in fish-eating wildlife species in contaminated areas such as the Baltic region. In a recent study in mink (*Mustela vison*), kit survival and body weight gain were reduced when mean PCB levels in muscle from the dams were 12 mg/kg l.w. (Brunström et al.. 1997). PCB concentrations in mature harbor porpoise *(Phocoena phocoena*) from the Baltic Sea sampled in 1988–1989 varied between 14 and 78 mg PCB/kg l.w. (Berggren 1995) and in Baltic grey seals levels varied between 32 and 5300 mg/kg l.w. in animals collected in 1979–1990 (Blomkvist et al., 1992). The median PCB level in otters (*Lutra lutra*) collected in southern Sweden in 1990–1994 was 45.5 mg/kg l.w., whereas the median level in animals from northern Sweden was 7.5 mg/kg l.w. (Mats Olsson, personal communication).

Birds

In 1957 millions of broilers died in south-eastern United States. The disease was due to toxic components in certain feed fats. Characteristic symptoms included the presence of excessive fluid in the heart sac and in the abdominal cavity. The toxic material in the fat was called chick edema factor, and the syndrome chick edema disease. Injection of extracts from toxic fat into chicken eggs resulted in decreased hatching and development of embryonic deformities, including beak and eye defects and edema. Over ten years after the outbreak of chick edema disease, the chick edema factor was identified as 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (Cantrell et al., 1969). Experimental studies have verified that Ah receptor agonists including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) are highly embryolethal and cause edema and deformities in chicken embryos (Higginbotham et al., 1968; Rifkind and Muschick, 1983; Brunström, 1989). There are very large species differences in sensitivity to these compounds and chicken embryos are considerably more sensitive than the other species tested (Brunström, 1988; 1989; Sanderson and Bellward, 1995; Kennedy et al., 1996).

Several species of colonial fish-eating birds nesting in the Great Lakes basin exhibit chronic impairment of reproduction (Gilbertson et al., 1991). High levels of DDT and its metabolites have caused eggshell thinning in many species. In addition to this, the reproductive impairment is characterized by high embryonic and chick mortality, edema, growth retardation, and deformities. This syndrome of fish-eating birds in the Great Lakes area has been designated GLEMEDS (Great Lakes embryo mortality, edema, and deformities syndrome) and clearly resembles another disease, the chick edema disease (see above). Reproductive impairment has been described

in a number of species including the double-crested cormorant (*Phalacrocorax auritus*), black-crowned night heron (*Nycticorax nycticorax*), herring gull (*Larus argentatus*), ring-billed gull (*Larus delawarensis*), common tern (*Sterna hirundo*), Forster's tern (*Sterna forsteri*), and Caspian tern (*Hydroprogne caspia*). Current concentrations of PCDDs/Fs and PCBs in fish-eating birds in the Great Lakes area are less than those during the 1960s and 1970s and some bird populations, such as double-crested cormorants and herring gulls have made dramatic recoveries since that time (Giesy et al., 1994). However, populations of common and Forster's tern continue to decline and double-crested cormorants and Caspian terns in Saginaw and Green Bays continue to display abnormally high rates of developmental deformities and embryolethality (Giesy et al., 1994).

No thorough investigation of the possible occurrence of GLEMEDS-like symptoms in fish-eating bird populations in the Baltic area has been carried out. The reproductive success of the white-tailed sea eagle (*Haliaeetus albicilla*) was negatively correlated to egg residue levels of DDE and PCB in eggs collected along the Baltic coast in 1965–1978 (Helander et. al., 1982). Bignert et al., (1995) studied eggshell thickness in Baltic guillemot (*Uria aalge*) eggs collected 1961–1989. They found that DDT and PCB levels have decreased in the Baltic since the 1970s and that the eggshell thickness has concomitantly increased.

Colonies of glaucous-winged gulls (*Larus glaucescens*) breeding in polluted areas of Puget Sound in 1984 exhibited eggshell thinning and persistent right oviducts in adult females (Fry et al., 1987). 21 of 31 female gulls had retained right oviducts. The causes of these effects in the glaucous-winged gulls are unknown. The major contaminants in Puget Sound were heavy metals, PCB and polycyclic aromatic hydrocarbons, whereas DDT concentrations were not particularly high.

Behavior

Hunt and Hunt (1973, 1977) noted female pairing in gulls in southern California when the sexes of birds having supernormal clutches were examined. Gulls normally lay 3 eggs but numbers of eggs in the supernormal clutches were 4–6. Supernormal clutches and female-female pairing have been noted in breeding populations of four species of gulls in North America. Western gull (*Larus occidentalis*) and herring gull (*Larus argentatus*) colonies with supernormal clutches have been located in areas contaminated with high levels of organochlorines. Since gulls are rather resistant to eggshell thinning by DDT (Peakall, 1975), levels of DDT that caused eggshell thinning in other species in southern California did not damage egg shells of gulls. Thus gull chicks hatched from eggs contaminated with high levels of organochlorines. Fry and Toone (1981) speculated that reproductive failures, skewed sex ratios, and female-female pairing in breeding populations of Western gulls on the Great Lakes resulted from organochlorine pollution. Feminization of male gull embryos was sug-

gested to have affected the reproductive behavior of the males leading to reduced migration to the breeding sites. An alternative hypothesis to explain the sex ratio skew in contaminated areas is that the low male/female ratio was due to a higher mortality among the males (Conover & Hunt, 1984). Altered incubation behavior resulting in decreased hatchability has been indicated in studies of herring gulls (Fox et al., 1978) and Forster's terns (Kubiak et al., 1989) in the Great Lakes area.

Reptiles

One of the best known cases of reproductive disturbance due to environmental endocrine disruption is in the alligators in Lake Apopka in Florida. The alligator males display a number of disturbances, including small penis size and suppressed plasma testosterone levels (Guillette et al., 1996). The testosterone levels in young male alligators in Lake Apopka are suppressed to levels normally found in females. Lake Apopka is a heavily polluted and eutrophic water and was also subject to a major industrial spill of difocol, DDT, DDE, DDD and sulfuric acid in 1980. Alligator eggs from Lake Apopka have been found to contain high levels of *p,p*'-DDE. The disruptive effects on reproduction were first suggested to be due to estrogenic contaminants, but later to the anti-androgenic effects of *p,p*'-DDE (Kelce et al., 1995). In tissue culture, 64 ppb of *p,p*'-DDE inhibited androgens from binding to the androgen receptor. In alligator eggs from Lake Apopka p,p'-DDE levels of 5800 ppb (lipid weight) have been observed (Guillette et al., 1996).

Fish

Vitellogenin

Vitellogenin production as a biomarker for estrogenic effects is being used to survey the status of rivers and lakes. In a British study, caged fish were placed in rivers and reservoirs to determine the estrogenicity of these waters (Purdom et al., 1994). Fifteen water reservoirs were studied but none of them elicited vitellogenin production in the caged fish. Six rivers were also studied. In five of these rivers, fish were caged downstream of sewage treatment plants. The sixth river was chosen since it received alkylphenol-containing water from a wool scouring industry.

A vitellogenic response could be detected in juvenile fish in all except one survey area (Purdom et al., 1994). However, the magnitude of response varied widely. In some river stretches vitellogenic responses could only be detected when fish were caged directly in the undiluted sewage effluent. On the other hand, in the river Aire, which received alkylphenolic water, the male juvenile fish produced vitellogenin at levels comparable to normal vitellogenic females. The exact cause of the estrogenic

response in these caged fish was not determined in these studies. However alkylphenols, nonylphenol ethoxylates and ethinyl estradiol were all suggested as possible inducers of the vitellogenic response.

When surveying the estrogenic response in caged fish along the river Lea, it was observed that there were elevated vitellogenin levels downstream of all major sewage plants. Since not all fish are stationary in these waters it is highly possible that many of the wild fish will be exposed to estrogenic effluents at some time during their life span.

Effluents from sewage treatment plants and textile factories have been shown to induce vitellogenin and lead to reduced testis size in caged fish.

M74 and the early mortality syndrome

Loss of co-ordination, irregular swimming and high mortality in newly hatched yolk-sac fry has been observed in the Baltic, in the North American Great Lakes and in the New York Finger Lakes. In the Baltic the syndrome, which is having a devastating effect on both feral hatchery-reared and on wild Baltic salmon, is known as M74 (Bengtsson and Hill, 1996). First in North America and subsequently in the Baltic area this syndrome has been connected to a lack of vitamin B1 (thiamin) (Marcquenski 1996; Amcoff et al., 1996), and the syndrome largely disappears if the eggs or the females to be stripped are treated with thiamin (Fitzsimons 1995; Fitzsimons and Brown 1996; Larsson and Haux 1996). The thiamin deficiency has been suggested to be due to thiaminase activity in clupeids which form the major part of salmonid food in these regions. The proportion of clupeids in the diet of salmonids may have increased (though actual data appear to be lacking) due to eutrophication of the Baltic leading to an increased plankton-production that favors clupeids and, in the North American lakes, to the introduction of clupeids. Eggs developing into M74 fry are paler than healthy eggs, due to a lower content of carotenoids, especially astaxanthin (Petterson and Lignell 1996) which is an effective antioxidant. Data from different years suggest, however, that the low carotenoid content in affected eggs is a symptom rather than a cause of the syndrome (Börjeson et al., 1994).

Environmental pollutants have often been assumed to be involved in the M74 syndrome as a primary or contributing cause. The case is, however, far from clear-cut.

Over the last decades, the general levels of PCBs and DDT in the Baltic have declined when M74 has increased. In the Great Lakes the levels of known organic contaminants have also decreased while the early mortality syndrome has increased (Fitzsimons and Brown, 1996). According to Marcquenski (1996) there are no indications for involvement of PCBs in the early mortality syndrome in North America and dieldrin, DDT and DDT- metabolites are practically absent in affected fry.

Norrgren et al., (1993) reported higher hepatic CYP levels and higher activities of the CYP1A catalyzed enzyme activity, ethoxyresorufin O-deethylase (EROD), in the liver of salmon females giving rise to M74 affected eggs than in females giving rise to healthy eggs in the river Mörrum. In contrast, Brown et al., (1996) found no difference in liver EROD and hydroperoxides between affected and unaffected lake trout (*Salvelinus namaycush*) in Lake Ontario, although the levels were higher than in the healthy Lake Manitou. EROD can be induced by numerous pollutants, including polycyclic aromatic hydrocarbons, PCBs and dioxins.

A number of studies have been carried out treating salmonids with defined xenobiotics or extracts. These include feeding of salmon with commercial pellets supplemented with Baltic herring oil (Bergqvist et al., 1994) and injection of eggs with extracts from affected eggs (Norrgren et al., 1993). Effects clearly related to M74 have not been observed.

To summarize, there is at present only weak evidence suggesting that these syndromes are related to xenobiotics. The mechanisms of their possible influence are not known and it is unclear if EDSs are involved.

Others

Reduced levels of testosterone have been observed for white sucker (*Catostomus commersoni*) collected downstream of a Kraft mill effluent (Munkittrick et al., 1991). Reduced gonad-size in female perch has been observed downstream of pulp mills in the Gulf of Bothnia and has been correlated to the distance from the effluent source (Andersson et al., 1988). The reduction in gonad-size was also correlated to an increase in EROD activity, indicating that the CYPIA gene was upregulated.

Masculinization of female fish has been observed in mosquitofish (*Gambusia affinis*) in a field study of Elevenmile Creek in Florida (Howell et al., 1980). The fish exhibited modification of the anal fin into a penis-like structure. It was found that these masculinized fish were all found downstream of a Kraft mill effluent. This effluent was found to contain large amounts of plant steroids including sitosterol and stigmastanol. The mechanism behind this response is not clear. Since the development of gonopodium in poecilid fishes is stimulated by androgens, especially 11-ketotestosterone (Schreibman et al., 1986) it does not fit with estrogenic actions

Imposex in molluscs

Unlike the pulmonate snails, most marine snails are normally not hermaphrodites. However, an imposed partial hermaphroditism in females, imposex, has been reported from several prosobranch marine snails (e.g. *Nucella, Buccinum, Littorina*) and has been observed in the Baltic sea. Imposex is characterized by non-

functional male genitalia in females. Imposex females develop a penis and a *vas deferens* as well as female reproductive organs. In severe cases the snails become sterile. Imposex has been connected with the presence of tributyltin, which is used in boat-paints to prevent fouling.

TBT levels from 20 ng/L suppress oogenesis and stimulate spermatogenesis in female *Nucella* (Gibbs et al., 988). Treatment of prosobranch snails with tributyltin at concentrations as low as 1 ng Sn/L ambient water induces imposex. Spooner (1991) found that TBT treatment increased the testosterone levels in *Nucella*, and that testosterone, like tributyltin, induced imposex.

TBT also induces spermatogenesis in oysters, though at a high concentration (240 ng/L) and at 2.6 µg/L it may also suppress gonad growth (Thain et al., 986).

In vitro exposure performed on periwinkle (*Littorina littorea*) microsomes has shown that even at exposure to 300 µg TBT/L there was only a 30–40% reduction in aromatase levels and that 29 mg TBT/L was needed to increase androstenedione formation from testosterone (Ronis and Mason, 1996). TBT had only modest effects on CYP-dependent (phase I) testosterone metabolism (Ronis and Mason, 1996). *In vivo* experiments were also performed to determine the levels of testosterone, its phase I metabolites as well as its conjugated forms (water soluble metabolites). It was found that non-conjugated steroid levels increased following 42h exposure to 2.9 mg TBT/L while there was a significant reduction in conjugation. These experiments indicate that TBT reduces aromatase activity to some extent, but that the main response is an inhibition of conjugation (phase II reactions). The inhibition of testosterone conjugation and accumulation of tissue androgens may be of importance for imposex development.

Effects of TBT on vertebrate reproduction have been studied to a limited extent and with largely negative results. Holm et al., (1991) found no consistent effects on reproduction in sticklebacks that had been exposed to up to 2.5 µg/L TBT oxide (nominal concentration) for 7.5 months. The significance of TBT for disturbances of vertebrate reproduction is therefore questionable.

TBT has many toxic effects on invertebrates and vertebrates apart from reproductive effects. It kills/damages larval stages of fish and invertebrates at relatively low concentrations. It causes deformation of bivalve shells, suppresses growth and EROD activity, and damages the nervous system and the immune-system.

The connection between TBT and imposex is well established and this and other negative environmental effects of tributyltin have led to its ban or use restriction in many countries, including Sweden.

SUMMARY

Several known or suspected impairments in human reproduction have been suggested to be the result of environmental pollutants. These impairments are consistent with effects of, especially, estrogenic compounds. However, as far as the general population is concerned (as opposed to groups particularly exposed due to accidents etc.) it is unclear whether, as in the case of declining sperm quality, the changes have actually occurred. It may be as in other cases, such as increased incidence of testicular cancer, that the observed changes are due to environmental pollutants (Table 4.1).

Neurobehavioural effects (e.g. impaired learning) of environmental pollutants have been indicated in exposed human populations and from laboratory studies. It is not known how widespread the phenomenon is or what the mechanism behind it are. Although the mechanisms remains to be investigated, neurological and intellectual parameters are important since they constitute sensitive endpoints in studies of perinatal exposure to environmental pollutants.

In wildlife, some reproductive effects have been correlated to exposure to environmental pollutants (Table 4.2). In land-living vertebrates the reproductive disorders appear primarily to have been induced by persistent organic pollutants, such as DDT, PCB and PCDD/F. These persistent compounds may also have caused disturbed reproduction in fish populations. In addition, reproductive markers in fish (mainly vitellogenin) have been shown to respond to substances that are readily metabolized and excreted, for example alkyl phenols. Biological responses indicative of estrogenic effects, such as induction of vitellogenin, have been observed in fish ex-

TABLE 4.1. Impairments in humans discussed in relation to endocrine disrupting chemicals

	Association with environmental pollutants
Neurobehavioural effects	probable *
Increased incidence of:	
– testis cancer	not shown
– prostate cancer	not shown
– breast cancer	not shown
– endometriosis	not shown
– hypospadias	not shown
– cryptorchidism	data on increased incidence not conclusive
Declined sperm quality	data on declined quality not conclusive

* Effects associated to intake of contaminated fish.

posed to less persistent pollutants without any documented influence on reproduction or population viability. All of the effects in wildlife have so far been observed in highly contaminated environments.

Most or all of the effects in humans and wildlife included in Tables 4.1 and 4.2 are consistent with experimental observations in animals exposed to organic pollutants. Many of these pollutants are well-documented modulators of endocrine functions (see Chapter 6). However, the mechanisms behind the observed effects in humans and wildlife are largely unknown and in most cases it is not clear whether or not the primary effect is a change in endocrine function.

TABLE 4.2. Impairments in wildlife discussed in relation to endocrine disrupting chemicals

Animal group	Effect	Association to environmental pollutants	
Mammals – panther	sperm quality cryptorchidism	probable	effects observed in inbred population
– mink, otter	population decrease	probable	
– seals	female reproductive disorders	yes	
	adrenocortical hyperplasia	yes	
Birds	eggshell thinning	yes	
	embryotoxicity/ malformations	yes	
	malformation of reprod. tract	probable	
	reproductive behavior	probable	
Reptiles – alligator	microphalli and lowered testosterone levels	probable	effects seen in connection to accidental contamination
Fish	vitellogenin	yes	
	masculinization	probable	
	lowered testosterone levels	probable	
	reduced testis size	probable	
	M74/EMS	not shown	
Molluscs	imposex	yes	

References

Adami, H., Bergström, R., Möhner, M., Zatonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., Gurevicius, R. and Stengrevics A. (1994) Testicular cancer in nine Northern European countries. Int. J. Cancer 59:33-38.

Ahlborg, U.G., Lipworth, L., Titus-Ernstoff, L., Hsieh, C.-C., Hanberg, A., Baron, J., Trichopoulos, D. and Adami, H.-O. (1995) Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: An assessment of the biological and epidemiological evidence. Crit. Rev. Toxicol. 24:463-531.

Amcoff, P., Norrgren, L., Börjesson, H., and Lindeberg, J.(1996) Lowered concentration of thiamine (vitamin B1) in M74-affected feral Baltic salmon (*Salmo salar*). In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:39-39.

Andersson, T., Förlin, L., Härdig, J. and Larsson, Å. (1988) Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. Can. J. Fish. Aquat. Sci. 45:1525-1536.

Ansell, P.E., Bennett, V., Bull, D., Jackson, M.B., Pike, L.A., Pike, M.C. Chilvers, C.E.D., Dudley, N.E., Gough, M.H., Griffiths, D.M., Redman, C., Willkinson, A.R., Macfarlane, A. and Coupland, C.A.C. (1992) Cryptorchidism: a prospecitve study of 7500 consecutive male births, 1984-8. Arch. Dis. Child. 67: 892-899.

Antunes, C.M., Rutledge, A., Pokempner, M. and Garcia, R. (1979) Endometrial cancer and estrogen use. Report of a large case-control study. N.Engl. J. Med. 300:9-13.

Auger, J., Kunstmann, J.M., Czyglik, F. and Jouannet, P. (1995) Decline in semen quality among fertile men in Paris during the past 20 years. N. Engl. J. Med. 332:281-285.

Barone, M.A., Roelke, M.E., Howard, J.G., Brown, J.L., Anderson, A.E. and Wildt, D.E. (1994) Reproductive characteristics of male Florida panthers: Comparative studies from Florida, Texas, Colorado, Latin America and North America. Zoos. J. Mammal. 75:150-162.

Begntsson, B.-E. and Hill, C. (1996) Review of recent research efforts on reporductive disturbances in Baltic fish. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:21-22.

Benvold, E. (1989) Semen quality in Norwegian men over a 20-year period. Int. J. Fertil. 34:401-404.

Berggren, P. (1995). Stocks, status and survival of Harbour porpoises in Swedish waters. Ph. D. Thesis, Department of Zoology, Stockholm University.

Bergqvist, P.-A., Andersson, T., Bengtsson, B.-E., Eriksson, T., Larsson, Å., Norrgren, L. (1994). A large-scale feeding experiment with fish extracts. In: L. Norrgren ed. Report from the Uppsala workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency. pp. 59-60.

Bergman, A. and Olsson, M. (1986). Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: Is environmental pollution the cause of a widely distributed disease syndrome? Finn. Game Res. No. 44:47-62.

Bignert, A., Litzen, K., Odsjö, T., Olsson, M., Persson, W. and Reutergårdh, L. (1995) Time-related factors influence the concentrations of sDDT, PCBs and shell parameters in eggs of Baltic guillemot (*Uria aalge*), 1861-1989. Environm. Poll., 89(1):27-36.

Blomkvist, G., Roos, A., Jensen, S., Bignert, A. and Olsson, M. (1992) Concentrations of sDDT and PCB in seals from Swedish and Scottish waters. Ambio 21:539-545.

Börjeson, H., Norrgren, L., Andersson, T. and Bergqvist, P.-A. (1994) The Baltic salmon - situation in the past and today. In: Report from the Uppsala workshop on reproduction disturbances in fish. (Norrgren, L. ed.) Swedish Environmental Protection Agency, Report 4346:14-25.

Bostofte, E., Serup, J. and Rebbe, H. (1983) Has the fertility of Danish men declined through the years in terms of semen quality? A compaarison of semen qualities between 1952 and 1972. Int. J. Fertil. 28:91-95.

Brown, S., Palace, V., Klaverkamp, J. and Fitzsimons, J. (1996) Vitamins and oxidative stress in lake trout (*Salvelinus namaycush*) displaying Early Mortality Syndrome. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:84-85.

Brunström, B., Lund, B.-O., Bergman, A., Asplund, L., Athanasiadis, I., Athanasiadou, M. and Asplund, L. (1997). Reproductive toxicity in mink (*Mustela vison*) chronically exposed to environmentally relevant PCB concentrations. Manuscript.

Brunström, B. (1988). Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. Poultry Science, 67:52-57.

Brunström, B. (1989). Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere, 19:765-768.

Cantrell, J.S., Webb, N.C. and Mabis, A.J. (1969) The identification and crystal structure of a hydrapericardium-producing factor: 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin. Acta Crystallogr., B25:150-156.

Carlsen, E., Giwercman, J.A., Kieding, N. and Skakkebaek, N.E. (1992) Evidence for decreasing quality of semen during past 50 years. B. Med. J. 305:609-613.

Chen, Y.C.,Yu, M.L., Rogan, W.J., Gladen, B.C. and Hsu, C.C. (1994) A 6-year follow-up of behavior and activity disorders in the Taiwan Yu-cheng children. Am. J. Publ. Health 84:415-421.

Cohen, C.J. and Rahaman, J. (1995) Endometrial cancer. Management of high risk and recurrence including the tamoxifen controversy. Cancer 76:2044-2052.

Comhaire, F., Van Waeleghem, K., De Clercq, N., Vermeulen, L and Schoonjans, F. (1995) Statement on the general reduction in sperm quality. Int. J. Androl. 2: 1-2.

Conover, M.R. and Hunt, G.L.Jr. (1984) Experimental evidence that female-female pairs in gulls result from a shortage of breeding males. Condor, 86:472-476.

Davis, D.L., Bradlow, H.L., Wollf, M., Woodruff, T., Hoel, D.G. and Anton-Culver, H. (1993) Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ. Health Perspect. 101:372-377.

Delong, R.L., Gilmartin, W.G. and Simpson, J.G. (1973) Premature births in California sea lions: Association with high organchlorine pollutant residue levels. Science, 181:1168-1170.

Facemire, C.F., Gross, T.S. and Guillette, L.J.J. (1995) Reproductive impairment in the Florida panther: nature or nurture. Environ. Health Perspect. 103 (Suppl.4): 79-86.

Fisch, H. and Goluboff, E.T. (1996) Geographic variations in sperm counts: a potential cause of bias in studies of semen quality. Fertil. Steril. 65:1044-1046.

Fisch, H., Goluboff, E.T., Olson, J.H.,

Feldshuh, J., Broder, S.J. and Barad, D.H. (1996) Semen analysis in 1,283 men from the United States over a 25-year period: no decline in quality. Fertil. Steril. 65:1009-1014.

Fitzsimons, J. (1995) The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. J. Great Lakes Res. 21:286-289.

Fitzsimons, J. and Brown, S. (1996) Effect of diet on thiamine levels in Great Lakes lake trout and relationship with Early Mortality Syndrome. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:76-78.

Fornander, T., Rutqvist, L.E., Cedermark, B., Glas, U., Mattsson, A., Silfverswärd, C., Skoog, L., Somell, A., Theve, T., Wilking, N., Askergren, J. and Hjalmar, M.L. (1989) Adjuvant tamoxifen in early breast cancer: occurence of new primary cancers. Lancet 1:117-120.

Fox, G.A., Gilman, A.P., Peakall, D.B. and Anderka, F.W. (1978) Behavioural abnormalities of nesting Lake Ontario herring gulls. J. Wildl. Manage., 42:477-483.

Fry, D.M., Toone, C.K., Speich, S.M. and Peard, R.J. (1987) Sex ratio skew and breeding patterns of gulls: Demographic and toxicologival considerations. Stud. Avian Biol., 10:26-43.

Gibbs, P.E., Pascoe, P.L. and Burt, G.R. (1988) Sex change in the female dogwhelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. J. Mar. Biol. Ass. U.K. 68:715-731.

Giesy, J.P., Ludwig, J. and Tillitt, D. (1994) Deformities in birds of the Great Lakes region. Assigning causality. Environ. Sci. Technol., 28:128-135.

Gilbertson, M., Kubiak, T., Ludwig, J. and Fox, G. (1991) Great lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. J. Toxicol. Environm. Health., 33:455-520.

Ginsburg, J., Okoo, S., Prelevic, G. and Hardiman, P. (1994) Residence in London area and sperm density. Lancet 343:230.

Grimvall, E., Rylander, R., Nilsson-Ehle, P., Nilsson, U., Strömberg, U., Hagmar, L. and Östman, C. (1997) Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation and fish consumption. Arch Environ Contam Toxicol 32:329-226.

Guillette, L.J. Jr., Pickford, D.B., Crain, D.A., Rooney, A.A. and Percival, H.F. (1996) Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. Gen. Comp. Endocrinol. 101:32-42.

Guo, Y.L., Lai, T.J., Ju, S.H., Chen, Y.C. and Hsu, C.C. (1993) Sexual development in biological findings in Yucheng children. DIOXIN 93:13th International Symposium on Chlorinated Dioxins and Related Compounds, 14:235-238.

Gusberg, S.B. (1947) Precursors of corpus carcinoma, estrogens, and adenomatous hyperplasia. Am. J. Obstet. Gynecol. 54:905-927.

Helander, B., Olsson, M. and Reutergårdh, L. (1982) Residue levels of organochlorine and mercury compounds in unhatched eggs and the relationships to breeding success in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. Holarct. Ecol., 5:349-366.

Helle, E., Olsson, M. and Jensen, S. (1976) PCB levels correlated with pathological changes in seal uteri. Ambio, 5:261-263.

Higginbotham, G.R., Huang, A., Firestone, D., Verrett, J., Rees, J. and Campbell, A.D. (1968) Chemical and toxicological evaluations of isolated and synthetic

chloro derivatives of dibenzo-*p*-dioxin. Nature, 220:702-703.

Holm, G., Norrgren, L. and Lindén, O. (1991) Reproductive and histopathological experimental exposure to bis(tributyltin) oxide (TBTO) on the three-spined stickleback, *Gasterosteus aculeatus Linnaeus*. J. Fish. Biol. 38:373-386.

Howell, W.M., Black, D.A. and Bortone, S.A. (1980) Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis* holbrooki: evidence for environmental-induced masculinization. Copeia 4:676-681.

Huisman, M., Koopman-Essenboom, C., Fidler, V., Hadders-Algra, M., van der Paauw, C.G., Tuinstra, L.G.M.T., Weisglas-Kuperus, N., Sauer, P.J.J., Touwen, B.C.L. and Boersma, E.R. (1995) Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Hum Dev 41:111-127.

Hunt, G.L. Jr. and Hunt, M.W. (1973) Clutch size, hatching success, and egg-shell-tinning in Western Gulls. Condor, 75:483-486.

Hunt, G.L. Jr. and Hunt, M.W. (1977) Female-female pairing in Western Gulls (*Larus occidentails*) in southern California. Science, 196:1466-1467.

Irvine, D.S. (1994) Falling sperm quality (letter). Br. Med. J. 309:476.

Jacobson, J.L., Jacobson, S.W. and Humphrey, H.E. (1990) Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J. Pediatr. 116:38-45.

Jacobson, J.L. and Jacobson, S.W. (1996) Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): the Michigan and North Carolina cohort studies. Toxicol. Ind. Health 12:435-445.

Jensen, T.K., Toppari, J., Keiding, N. and Skakkebaek, N.E. (1995) Do environmental estrogens contribute to the decline in male reproductive health? Clin. Chem. 41, 1896-1901.

Joffe, M. (1996) Decreased fertility in Britain compared with Finland. *Lancet* 347, 1519-1522.

Kelce, W. R., Stone, C. R., Laws, S. C., Gray, L. E., Kemppainen, J. A. and Wilson, E. M. (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature. 375: 581-585.

Kennedy, S.W., Lorenzen, A., Jones, S.P., Hahn, M.E. andStegeman, J.J. (1996) Cytochrome P4501A induction in avian hepatocyte cultures: A promising approach for predicting the sensitivity of avian species to toxic effects of halogenated aromatic hydrocarbons. Toxicol. Appl. Pharmacol., 141:214-230.

Koopman-Essebom, C., Huisman, M., Weisglas-Kuperus, N., Boersma, E.R., de Ridder, M.A., Van der Paauw, C.G., Tuinstra, L.G. and Sauer, P.J. (1994) Dioxin and PCB levels in blood and human milk in reltion to living areas in the Netherlands. Chemosphere 29:2327-2338.

Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E. and Erdman, T.C. (1989) Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan, 1983. Arch. Environ. Contam. Toxicol., 18:706-727.

Larsson, D.G.J. and Haux, C. (1996) Possible roles of thiamine, thiaminases and other thiamine inactivating factors in the EMS/M74 syndrome. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:40-41.

Lilienthal, H. and Winneke, G. (1991) Sensitive periods for behavioral toxicity of polychlorinated biphenyls: determina-

tion by cross-fostering in rats. Fundam. Appl. Toxicol. 17:368-375.

Marcquenski, S.V. (1996) Characterization of Early Mortality Syndrome (EMS) in salmonids from the Great Lakes. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:39-39.

Munkittrick, K.R., Miller, P.A., Barton, D.R. and Dixon, D.G. (1991) Altered performance of white sucker populations in the Manitouwadge chain of lakes is associated with changes in benthic macroinvertebrate communities as a result of copper and zinc contamination. Exotoxicol. Environ. Saf. 21:318-326.

Norrgren, L., Andersson, T., Bergquist, P.-A. and Björklund, I. (1993) Chemical, physiological and morphological studies of feral Baltic salmon (*Salmo salar*) suffering from abnormal fry mortality. Environ. Toxicol. Chem. 12:2065-2075.

O'Brien, S.J. (1994) A role for molecular genetics in biological conservation. Proc. Natl. Acad. Sci. U.S.A. 91:55748-5755.

Olsen, G.W., Bodner, K.M., Ramlow, J.M., Ross, C.E. and Lipshultz, L.I. (1995) Have sperm counts been reduced 50 percent in 50 years? A statistical model revisited. Fertil. Steril. 63:887-893.

Olsson, M., Johnels, A.G. and Vaz, R. (1975). DDT and PCB levels in seals from Swedish waters. The occurrence of aborted seal pups. Proceeding from the symposium on the seal in the Baltic. SNV PM, 591:43-65.

Paulsen, C.A., Berman, N.G. and Wang, C. (1996) Data from men in greater Seattle area reveals no downward trend in semen quality: further eivdence that deterioration of semen quality is not geographically uniform. Fertil. Steril. 65: 1015-1020.

Peakall, D.B. (1975) Physiological effects of chlorinated hydrocarbons on avian species. In: Environmental dynamics of pesticides (Hauge, R. and Freed, V.H. eds.). Plenum Press, New York. 343-360.

Petterson, A. and Lignell, Å. (1996) Decreased astaxanthin levels in the Baltic salmon and the M74 syndrome. In: Report from the Second Workshop on Reproduction Distrubances in Fish. (Bengtsson, B.-E., Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534:28-29.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R. and Sumpter, J.P. (1994) Estrogenic effects of effluents from sewage treatment works. Chem. Ecol. 8:275-285.

Reijnders, P.J.H. (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature, 324:456-457.

Rifkind, A.B. and Muschick, H. (1983). Benoxaprofen suppression of polychlorinated biphenyl toxicity without alteration of mixed function oxidase function. Nature 303:524-526.

Rogan, W.J, Gladen, B.C., McKinney, J.D., Carreras, N., Hardy, P., Thullen, J., Tingestad, J and Tully, M. (1986) Neonatal effects of transplacental exposure to PCBs and DDE. J. Pediatr. 109:335-341.

Rogan. W.J, Gladen, B.C., Hung, K.L., Koong, S.L., Shih, L.Y., Taylor, J.S., Wu, Y.C., Yang, D., Ragan, N.B. and Hsu, C.C. (1988) Congenital poisoning by polychlorinated biphenyls and their contaminats in Taiwan. Science 241:334-336.

Ronis, M.J.J. and Mason, A.Z. (1996) The metabolism of testosterone by the preiwinkle (*Littorina littorea*), in vitro and in Vivo: Effects of tributyl tin. Mar. Environ. Res. 42:161-166.

Rubin, G.L., Peterson, H.B., Lee. N.C., Maes, E.F., Wingo, P. and Becker, S. (1990) Estrogen replacement therapy and the risk of endometrial cancer: remaining controversies. Am. J. Obstet. Gynecol. 162:148-154.

Rylander, L., Strömberg, Ul and Hagmar, L. (1995) Decreased birthweigth among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine componuds. Scand. J. Work Environ. Health 21: 368-375.

Rylander, L., Strömberg, U. and Hagmar, L. (1996) Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birthweight. Scand. J. Work Environ. Health 22:260-266.

Rylander, R., Dyremark, E., Strömberg, U., Östman, C., Nilsson-Ehle, P. and Hagmar, L. (1997) Polychlorinated biphenyls in blood plasma among Swedish female fish consumers in relation to low birthweight. Submitted.

Sanderson, J.T. and Bellward, G.D. (1995). Hepatic microsomal ethoxyresorufin O-deethylase-inducing potency *in ovo* and cytosolic Ah receptor binding affinity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Comparison of four avian species. Toxicol. Appl. Pharmacol., 132:131-145.

Schreibman, M.P., Margolis-Nunno, H., Halpern-Selbold, L.R., Goos, H.J.Th. and Perlman, P.W. (1986) The influence of androgen administration on the structure and function of the brain-pituitary-gonad axis of sexually immature platyfish, *Xiphophorus maculatus*. Cell Tissue Rec. 245:519-524.

Scorer, C.G. (1964) The decent of the testis. Arch. Dis. Childh. 39:605-609.

Seoud, M.A., Johnson, J. and Weed, J.C. Jr. (1993) Gynecologic tumorss in tamoxifen-treated women with breast cancer. Obstet. Gynecol. 82:165-169.

Sharpe, R.M. and Skakkebaek, N.E. (1993) Are oestrogens involved in failing sperm counts and disorders of the male reproductive tract? Lancet 341:1392-1395.

Skakkebaek, N.E. and Keiding, N. (1994) Changes in semen and the testis. Br. Med. J. 309:1316-1317.

Spooner, N., Gibbs, P.E., Bryan, G.W. and Goad, L.J. (1991) The effects of tributyltin upon steroidtitres in the female dogwhelk, *Nucella lapillus*, and the development of imposex. Mar. Environ. Res. 32:37-49.

Suominen J. and Vierula M. (1993) Semen quality of Finnish men. Br. Med. J. 306: 1579.

Suzuki, K., Ito, K., Tamura, Y., Suzuki, T., Honma, S. and Yamanaka, H. (1996) Effects fo aromatase inhibitor, TZA-2209, on the prostate of androstenedione-treated castrated dogs: changes in prostate volume and histopathological findings. Prostate 28:328-337.

Thain, J.E., Waldock M.J., Helm M. 1986. The effect of tri-butyl-tin on the reproduction of the oyster *Ostrea edulis*. International Council for the Exploration of the Sea, CM 1986/E:14. p 1-8.

W.H.O. (1991) Congenital Malformations Worldwide: A report from the International Clearinghouse for Birth Defects Monitoring Systmens. p.113-118. Elsevier, Oxford.

5. Environmental contaminants with demonstrated or discussed hormonal effects

A number of organic compounds, or groups of compounds, primarily aromatic compounds, with potential hormone modulating effects are presented below. Metals and inorganic compounds are not included. Some chemico-physical data are given and their concentrations in the environment and in humans are briefly reviewed. The selected compounds are mainly those that have been reported to have endocrine disrupting capacity but a few compounds lacking such data have been included because of their presence in the environment and structural similarity to hormones or to other endocrine disrupting compounds. In addition, a few compounds are briefly presented which are persistent but for which data on toxicity and environmental levels are scarce. The present document includes only those pesticides which are persistent, have reported hormonal modulating capacity and are present in the Swedish environment. Several other pesticides that may be relevant, as well as many other substances, are not included in this document but are reviewed by Toppari et al., 1996. Persistent organic compounds have been presented in reports such as Swedish EPA Report 4563 and the reader is referred to these for further information on use and regulations.

The use, or emission, of some of the presented compounds is regulated (e.g. PCB, DDT, Lindane, PCDD, PCDF) in order to minimize the exposure in the environment. However, a majority of the here described environmental contaminants, and for which concern has been raised, are not yet regulated (e.g. PBDE, PCN, nonylphenol, halogenated benzenes and phenols, brominated and non-brominated bisphenol A).

Most of the compounds presented below are persistent and biomagnifying substances that may undergo metabolic transformations, forming metabolites that may either be water soluble, and therefore excreted, or are lipophilic enough to be retained in the organism. Compounds or their metabolites may, depending on their structure, bind to proteins. Both estradiol and thyroxine have a phenolic group and this is also a structural element in metabolites formed from most aromatic compounds. It is thus of importance to include a brief discussion of the metabolism of the compounds. As shown, the concentration of the metabolites may occasionally be in the same range as the parent compounds. The exposure of an organism for a compound, or its metabolites, is related to the concentration and half-lives of the compound in the body. The kinetics of persistent and semi-persistent compounds must therefore be considered in the risk assessment for interactions with hormone systems in the living organism.

General metabolism

A simplified scheme of the metabolism of aromatic compounds is shown in Figure 5.1 using benzene as a model for halogenated aromatic compounds. The first step, creating a functional group is catalysed by the CYP enzyme family, and often results in an arene oxide. This is a reactive intermediate, which may react with endogenous macromolecules but is mostly rearranged to a phenol. The phenolic, also called hydroxylated, metabolite can be further metabolized by conjugation to sulphates or glucuronic acid. As conjugates, the water solubility is increased and the compounds can be excreted. Some xenobiotics, i.e. PCBs and chlorinated benzenes, may also be metabolised via the mercapturic acid pathway (MAP). This involves a reaction between glutathione (GSH) and the arene oxide, subsequently resulting in the formation of water soluble mercapturic acids or methyl sulphides and/or methyl sulphones. The two latter types of metabolites are lipophilic and are therefore not easily excreted. Aryl methyl sulphones are important metabolites and examples of their toxic effects are given in Chapter 6.

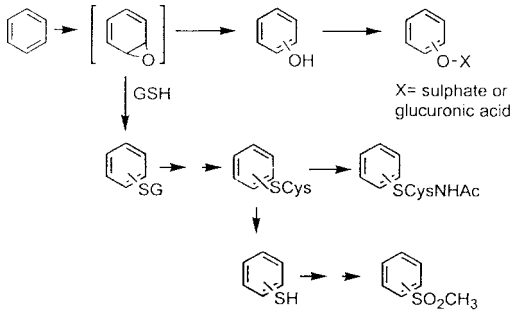
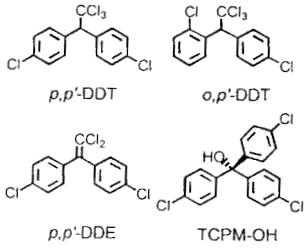


Figure 5.1. Simplified metabolism scheme for aromatic compounds.

PESTICIDES

Many pesticides are reported to be endocrine disruptors, and effects on the thyroid are frequently reported. However, high doses are often required to obtain effects and the no observed effect level (NOEL) is far above the acceptable daily intake (ADI) and above the estimated daily intake (Toppari et al., 1996). Most of the pesticides reported to be endocrine disruptors are banned in Sweden, although some are persistent and are still present in the environment, or are deposited in Sweden after long range transport (eg. DDT and Lindane). An interesting group of pesticides is the so-called hormone-type, often acting as growth hormone, or inhibiting growth hormones (insecticides). Examples from this group are phenoxy acetic acids and benzoic acid herbicides, and methoprene (insecticide).

DDT and related compounds



p,p'-DDT(1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane) has been banned in Sweden for more than 20 years but is still produced for pesticide use in some countries. In the production process *o,p'* -DDT is also formed as a byproduct (15–20%) and in addition, trace amounts (ca 100 ppm) of tris(4-chlorophenyl) methane (TCPM) is formed (Buser et al., 1995). Tris (4-chlorophenyl) methanol (TCPM-OH) has been suggested to be a metabolite of TCPM and/or a pollutant from production of optical active polymers (Walker et al., 1989, Buser et al., 1995). TCPM-OH has also been produced industrially.

DDT is metabolized to 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE) and 1,1-dichloro–2,2-bis(4-chlorophenyl)ethane (DDD) and further to di(chlorophenyl) acetic acid (DDA). However, the major metabolite is DDE, which is highly persistent. Hydroxylation on the phenyl rings occurs and such metabolites have also been determined in e.g., seal faeces (Jansson et al.,1975). DDE is further metabolized via the MAP, as exemplified by the formation of methyl sulphonyl metabolites of DDE (MSF-DDE), which are ubiquitous contaminants (Letcher et al., 1995).

In environmental samples, the concentration of DDT is often given as sDDT, which is the sum of DDT, DDE and DDD. *o,p*'-DDT, known as an endocrine disrupting compound, is rarely analysed separately, if at all, because the concentrations are much lower (often <1%) compared to sDDT or DDE (Jansson et al., 1993; Lundén and Norén, 1998). Generally, in biota, the most abundant compound is *p,p'* -DDE and the relative amount of *p,p'* -DDT present is an indication of how recently DDT was released.

Environmental levels

The levels of *p,p*'-DDE and total DDT in Baltic herring, ringed seal and human milk are shown in Figures 5.2, 5.3 and 5.4. In analysis of *p,p*'- and *o,p*' -DDT in different Swedish species, the *o,p*'-DDT level was below the detection limit except in Skagerrak and Baltic herring, where the *o,p*'-DDT levels were 50–500 times lower (10 and 21 ppb lipid weight (l.w), respectively) than *p,p*'-DDT (Jansson et al., 1993). Unusually high concentrations of *o,p*'-DDT compared to *p,p*'-DDE were determined in the tissues of fin whales (*Balaenoptera physalus*). The *p,p*'-DDE to *o,p*'-DDT ratio was only ca 1.5. The total concentrations were low, 260 ppt l.w. as were the PCB levels, ca 1 ppb l.w. (Aquilar and Borrell, 1994). Interestingly, *o,p*'-DDT was determined only in the brain, but not in muscle, liver or kidney from bald eagles from Lake Superior. The *o,p*'-DDT concentrations were 0.05 and 0.18 ppm wet weight, respectively, in the two analysed samples, whereas the *p,p*'-DDE concentrations were 1.5 and 16 ppm (Kozie and Anderson, 1991).

The sDDT-levels in Swedish human milk (Stockholm mothers' milk Centre) has decreased about 10-fold since 1972 (Figure 5.5, Lundén and Norén, 1998). The *o,p*'-DDT levels were only determined in human milk samples from 1990–1992 and were between 2 and 4 ppb in those samples. The rapid decline makes it difficult to compare the concentrations in other human samples but it is interesting to note that the concentrations of sDDT in Mexico are as high as 14,000 ppb l.w. (mean value, range 500–162,000 ppb l.w.; Waliszewski et al., 1995).

In Swedish mother's milk, MSF-DDE has been determined and the concentration in the pooled sample from 1992 was 0.46 ppb l.w. (Figure 5.4). The MSF-DDE to DDE ratio was consistently 0.002 in all samples (Norén et al., 1996).

The contaminant TCPM, and the structurally related TCPM-OH, are both persistent and bioaccumulating, as indicated by the high concentrations of these compounds in biota at higher trophic levels. TCPM-OH was first identified and reported in blubber from the harbour seal (*Phoca vitulina*) in Puget Sound at concentrations ranging from 23 to 750 ppb l.w. (Walker et al., 1989). TCPM-OH has since then been shown to be globally distributed and in one of the most highly polluted areas, the mean concentration of TCPM-OH in peregrine falcon eggs from British Columbia was 1,100 ppb l.w., TCPM 2000 ppb and the sDDT concentration 1,600,000 ppb (Jarman et al., 1992).

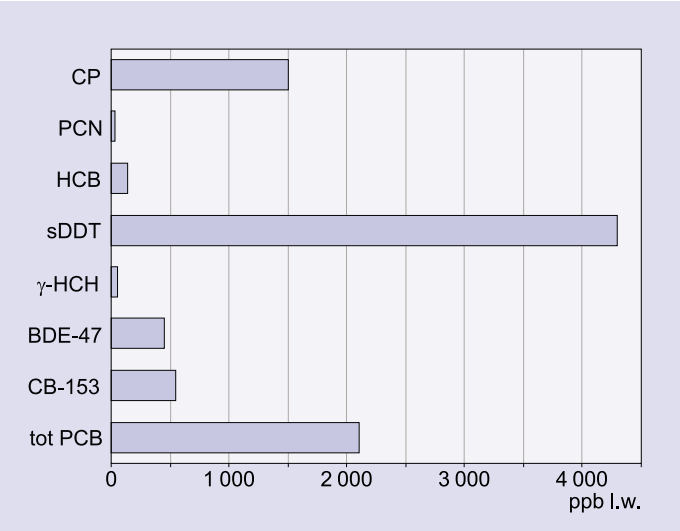


Figure 5.2. Some environmental pollutants in Baltic herring. Data from Jansson et al., 1993.

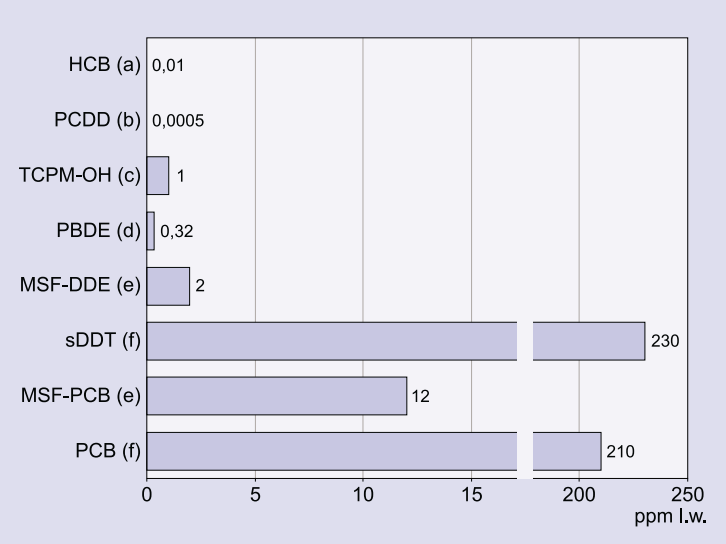


Figure 5.3. Environmental contaminants in Ringed seal blubber. All data but the HCB are from the same seal material. Data from a = Jansson et al., 1993, b = Bergek et al., 1992; c = Zook et al., 1992; d = Andersson and Wartianen 1992; e = Haraguchi et al., 1992; f = Blomkvist et al., 1992.

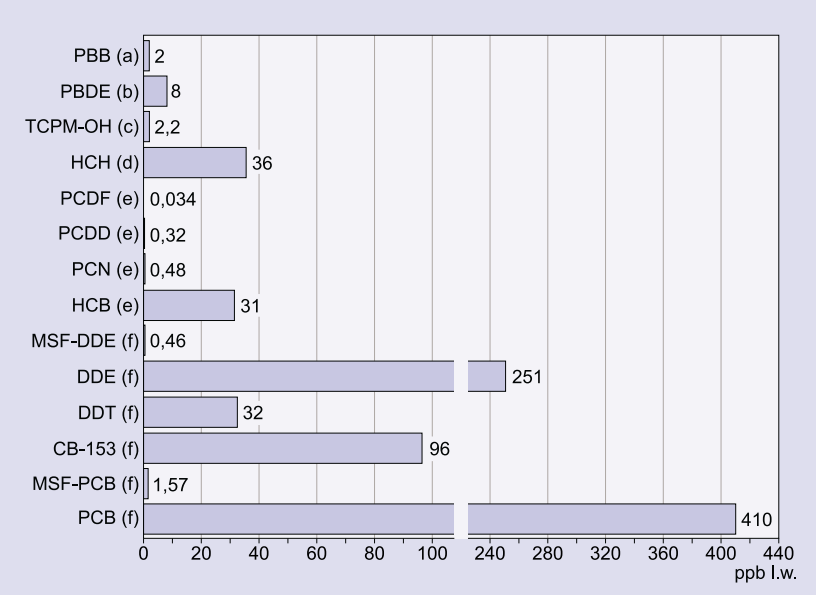


Figure 5.4. Levels (ppb lw) of environmental pollutants determined in mothers' milk. Data from a = Krüger et al., 1988; b = Krüger 1988; c = Johansen et al.,1994; d = Rahman et al., 1993 (Swedish milk samples); e = Norén *et al* 1996; f = Lundén and Norén 1998.

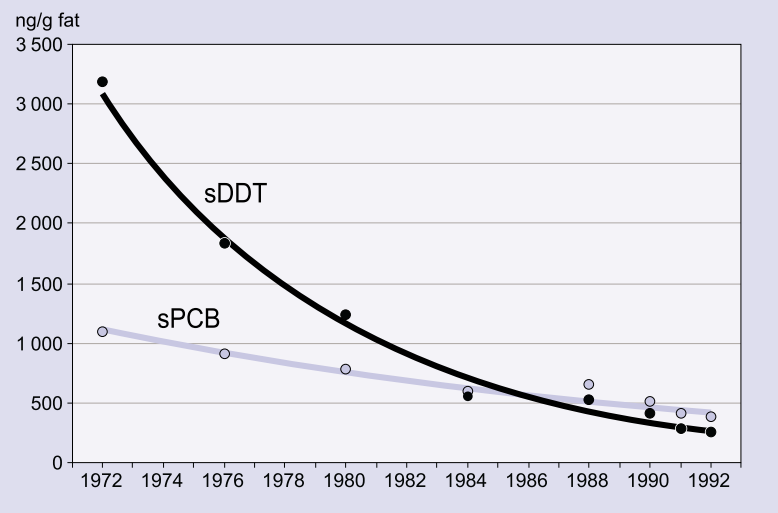
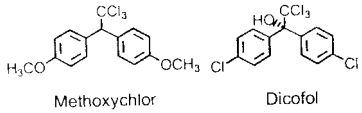


Figure 5.5. Time trend of PCB and sDDT in human milk (ppb l.w.) from 1972 to 1992. Data from Lundén and Norén, 1998.

In polar bear liver, the TCPM-OH concentration was 4,000 ppb l.w., which was only 2.5 times lower than the sDDT (10,000 ppb) (Jarman et al., 1992). TCPM-OH has been determined, e.g., in Baltic ringed seal liver (3,000 ppb l.w.) and blubber (ca 1,000 ppb l.w.) (Figure 5.3). TCPM has been determined in some Dutch eel samples at low ppb levels (de Boer et al., 1994).

TCPM-OH is also present in Swedish breast milk at low concentrations, 2–3 ppb l.w. (The PCB concentrations in these samples were 224±80 ppb and sDDT 650±370 ppb l.w.) (Rahman et al., 1993) (Figure 5.4). There was no correlation between the TCPM-OH and other organochlorine contaminants. The origin of TCPM-OH is still not clarified.

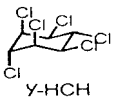
Methoxychlor and Dicofol



Methoxychlor Dicofol

These two compound were introduced as pesticides to replace DDT and are structurally similar. Both compounds are now banned in Sweden but are still used in many countries, including some EU-countries. Dicofol is produced from DDT and the final product may contain DDT as a contaminant. Methoxychlor is metabolically demethylated and forms hydroxylated derivates that are structurally similar to hydroxylated DDT-metabolites.

γ-Hexachlorocyclohexane (γ-HCH, Lindane)



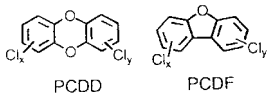
γ-HCH

An example of a pesticide that has been studied for endocrine effects is γ-HCH, also called Lindane. In the technical product other isomers are also present but γ-HCH is the most active as an insectide. While the purified γ-HCH was used in Sweden, the crude product is still often in use in developing countries. The β-HCH isomer is the most persistent and both α- and β-HCH reach Sweden by long-range transport. The HCHs are lipophilic and persistent and can biomagnify in food webs.

Lindane, which is not used in Sweden but in most EU-countries, is transported to Sweden by long-range transport and during the spraying season, the concentrations of Lindane in rain water increase in Sweden (Kreuger, 1995). β-HCH and α-HCH, are more persistent than γ-HCH and are bioaccumulated in the food webs (Jansson et al., 1993). In Italian human milk, β-HCH was 180 ppb l.w. and in Norwegian human milk, a concentration of 33 ppb l.w. was determined (Figure 4.4; Larsen et al., 1994; Johanssen et al., 1994). In adipose tissue from Iran the concentration of β-HCH was 730 ppb l.w. and of α-HCH 18 ppb (Burgaz et al., 1995).

HALOGENATED INDUSTRIAL CHEMICALS AND UNINTENTIONALLY FORMED BYPRODUCTS

PCDD and PCDF



PCDD PCDF

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are groups of lipophilic and persistent compounds, that are unintentionally formed during combustion (e.g. municipal waste incineration), metal production and reclamation, production of pulp and paper, chlorophenols, and chlorinated phenoxy herbicides, and at chlorine-alkali plants using graphite electrodes, (Rappe

1994). In addition, PCDD has been shown to be formed enzymatically in composts and sewage sludge (Öberg et al., 1990, 1992 and 1993).

PCDD consists of 75 possible congeners and PCDF of 135 congeners. The relative amounts of the PCDD and PCDF congeners vary with production and the congener pattern can therefore be used to identify a source (Rappe, 1994). Both groups of compounds are highly hydrophobic and log K_{OW} in the range of 6 to 9 for tetra- to octaCDD have been reported (Götz et al., 1994).

Brominated dibenzo-*p*-dioxin and dibenzofurans are formed during combustion of, e.g., polymers containing brominated flame retardants.

The most toxic PCDD congener is 2,3,7,8-TCDD, and it is the standard of toxicity. The toxic equivalence factor (TEF) for other compounds is set as the potency of the compound/the potency of 2,3,7,8-TCDD. The concentrations of PCDD/Fs are often presented as the sum of the concentration multiplied with the TEF, yielding toxic equivalents (TEQ s) in the sample.

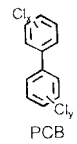
Metabolism

PCDD/Fs are metabolized at different rates, depending on substitution pattern. Thus, 2,3,7,8-substituted congeners are more persistent than other congeners, as indicated by their relative persistence in food chains (van den Berg et al., 1987; Ryan et al., 1985). Metabolism occurs via arene oxide formation and hydroxylated metabolites have been determined for at least tetrachlorinated congeners (Larsen et al., 1996). In addition, cleaving of the ether bridges between the two phenyl rings were shown to occur resulting in catechols.

Environmental levels

Geographically, the PCDD/Fs are of approximately the same level in herring at different locations along the Swedish east coast (150 ppt TEQ l.w.) but are lower in herring from the west coast (24 ppt TEQ l.w. in 1994). The levels of PCDD/Fs are very low in terrestrial species (de Wit et al., 1994). Chemically, levels of total PCDD/F s in herring from the Baltic have been reported to be around 700 ppt l.w. The levels in seals are considerably lower, 11 and 50 ppt in ringed seal and grey seal respectively (Asplund et al., 1990). The levels in fish-eating birds is much higher, 1,100 and 2,700 ppt in guillemot and sea eagle respectively, but the levels have decreased about five times from 1972 to 1992 (de Wit et al., 1994). In Figures 5.3 and 5.4, examples of PCDD concentrations in ringed seal blubber and human milk are shown.

PCB



Polychlorinated biphenyls (PCBs) were produced for use as flame retardants and plasticizers with a wide range of applications, such as in capacitors, heat-exchangers, transformers, hydraulic oils, sealants, dedusting agents, paint and self-copying paper. They were produced by chlorination of biphenyl which resulted in products consisting of many individual chlorinated biphenyls (CBs). Theoretically, 209 CBs can be formed but in technical mixtures ca 130 CBs have been identified. CBs are highly hydrophobic with log K_{OW} ranging from 4 up to 8 (Rapaport and Eisenreich, 1984). They are persistent to degradation in the physical environment compared to in biota where biotransformation of certain CBs may be rapid. The structure determines the biological transformation rate and is also highly relevant to potential toxicity, with features such as number of *ortho*-chlorine atoms and presence of adjacent unsubstituted *meta*-/*para*-positions as the most important. (Figure 5.6).

PCBs are metabolised both to hydroxylated metabolites (OH-PCBs) and methyl sulphonyl metabolites (MSF-PCBs) (Figure 5.7). Both types of metabolites can, depending on structure, be selectively retained in organisms. Certain OH-PCBs are retained in plasma because they have a structural similarity to thyroxine and therefore compete with thyroxine for a carrier protein, transthyretin (Lans et al., 1993). The OH-PCBs have been determined in the blood of humans as wells as in several wild-life species and the concentrations of these OH-PCBs are for instance in human plasma, 10–50% of the PCB concentration (Bergman et al., 1994a). Depending on structure, MSF-PCBs may bind to proteins in different tissues, and have also been determined in several wild-life species, e.g. seals, as well as in human milk (Figures 5.3 and 5.4).

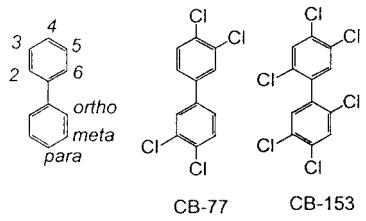


Figure 5.6. Structure of PCB, position nomenclature and two examples of PCB congeners i 3.3'4.4'-tetra-chlorobiphenyl (CB-77) and 2.2'4.4'5.5'-hexachlorobiphenyl (CB-153) (Ball-Schmiter et al., 1993).

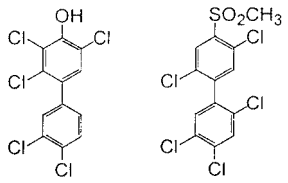


Figure 5.7. Examples of hydroxylated and methylsulphonyl-substituted PCB metabolites.

PCBs are still of major concern for the society due to high concentrations in biota compared to other environmental contaminants.

Polybrominated biphenyls (PBBs) are brominated analogues of PCBs and are still used as flame retardants. The chemical and physical properties are similar to those of PCBs although brominated analogues are more lipophilic. The congener pattern of the technical products consists of fewer congeners than technical PCB mixtures and in lower brominated products than the decaBB, the 2,2',4,4',5,5'-hexaBB is the major component (Ballschmiter et al., 1989). DecaBB is still used as a flame retardant and it can not be excluded that this compound is abiotically transformed to lower brominated compounds.

Metabolism of PCBs

Chlorinated biphenyls are mainly metabolised via arene oxides but direct insertion of hydroxy-groups also occurs (Preston et al., 1983; Koga et al., 1995). The metabolic rate depends on the substitution pattern so that CBs having unsubstituted adjacent *meta*-/*para*-positions are more susceptible to metabolism than those having chlorine atoms in one or both positions. CBs with the latter type of substitution pattern are those dominating in species at higher trophic levels. Thus 12 congeners constitute ca 80% of the total PCB concentration in human adipose tissue (Borlakoglu and Haegele 1991). The half-life of higher chlorinated PCB congeners (corresponding to Aroclor 1254) has been calculated to be 4.8 years in occupationally exposed workers (Phillips et al., 1989).

PCBs are converted to phenolic metabolites, which in general are conjugated and excreted, but may also form MSF-PCBs via the mercapturic acid pathway (Bakke et al., 1982, Bergman and Klasson Wehler, 1996, Klasson Wehler et al., 1996). CBs with a chlorine atom in a *para*-position can also be converted to 1,2-shifted hydroxylated metabolites (Yoshimura et al., 1987; Klasson Wehler et al., 1989; Koga et al., 1992; Klasson Wehler et al., 1993; Ariyoshi et al., 1995). In addition to being hydrophobic and bioaccumulating, many of the MSF metabolites are selectively retained in tissues, due to protein binding (Bergman et al., 1979; Brandt and Bergman 1987; Bergman et al., 1994b; Bergman and Klasson Wehler 1996).

Environmental levels

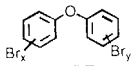
PCBs are found at all trophic levels in the environment and they are biomagnified in the food chains (Jansson et al., 1993; Norstrom et al., 1988; Kannan et al., 1995). In the Swedish environment, PCB levels are regularly monitored and in e.g. guillemot eggs from the Baltic area, the levels have dropped approximately 4 times from 1971 to 1995 (Bignert et al., 1995).

Reported values for human adipose tissue vary from 150 to 1400 ppb l.w. (Tanabe et al., 1993; Luotamo, et al., 1991), plasma ca 1,100 ppb l.w. (from Swedish men with a normal consumption of fish, (Asplund et al., 1994) and in human milk. The total PCB concentrations in human milk has decreased from 1,100 ppb l.w. in 1972 to 380 ppb in 1992 (Lundén and Norén, 1998) (Figure 5.5). The latter concentration is similar to the levels found in a Norwegian study, where mothers giving birth to their first child had a mean level of 372 ppb l.w. in milk samples collected during the first days of lactation (day 3–5) (Johansen et al., 1994).

MSF-PCBs are present in various tissues from different species in the environment, such as in seals from Swedish coastal waters (Figure 5.3) (Haraguchi et al.,1992; Bergman and Klasson Wehler 1996) and in seal and polar bears from the Arctic (Letcher et al., 1995). In human milk, MSF-PCB metabolites have been reported at levels of 0.5–2 ppb l.w. (Norén et al., 1996) (Figure 5.4). Also OH-PCBs have been determined in human milk, the same congeners as observed in plasma, but quantitative data was only given for one congener 4-OH-2,3,5,6,2',4',5'-heptaCB (1.9 pg/g l.w.) (Newsome and Davies, 1996).

2,2',4,4',5,5'-HexaBB has been found in biota although at lower levels than most PCB congeners (e.g. 0.29 ppb l.w. in herring from Skagerack and 0.16 ppb in Baltic herring; Grey seal blubber 26 ppb (Jansson et al., 1993)). PBB has also been determined in German mothers milk at a concentration of 2 ppb l.w. (mean for 25 women; Krüger et al., 1988).

Polybrominated diphenyl ethers



PBDE

Polybrominated diphenyl ethers (PBDEs) are produced at approximately 30000 ton/year and used as flame retardants in plastic components used in electronics but also in textiles. Technical mixtures consist of several PBDE congeners, with 4 up to 10 bromine atoms. The PBDEs are lipophilic with calculated log K_{OW} ranging from 5 to 11 (di-to decaBDE) and have been shown to bioaccumulate (Jansson et al., 1993; Pijnenburg et al., 1995; Sellström, 1996). The PBDEs are present in environmental samples, such as herring, seal and guillemot (Figure 5.8; Jansson et al., 1993) but also in human plasma (Klasson Wehler et al., 1997). PBDEs can be debrominated by UV-light and can also form brominated dibenzofurans (Watanabe and Tatsukawa, 1987). Half lives for PBDE congeners in rats have been reported to range from 19 to 129 days (tetra to hexaBDE) (von Meyerinck et al., 1990), indicating a slow metabolism. In contrast, decaBDE has been reported to be rapidly metabolized and excreted in the rat (ElDareer et al., 1987).

Metabolism

Few metabolism studies have been performed with halogenated diphenyl ethers. Generally, the most common metabolic route seem to be hydroxylation, whereas ether bond scission seems to be a minor route (Becker et al., 1991).

^{14}C-Labelled decaBDE is metabolized by the rat, and forms at least 3 metabolites that are more polar than the parent compound. At 72 h after the iv dose, 74% of the dose was present in faeces and gut contents, as determined by radioactivity, and 63% of the excreted faecal radioactivity corresponded to metabolites (El Dareer et al., 1987). These data indicate that decaBDE is rapidly metabolised and excreted. In contrast, 2,2',4,4'-tetraBDE is metabolised and excreted very slowly by the rat. Only 15% of an oral dose was excreted (primarily via faeces) within 5 days, mainly as parent compound. In this study, metabolism in the mouse was studied in parallel and the mouse excreted 20% via faeces (mainly as parent compound) and 30% via urine (as unidentified metabolites). For both species, the tissues contained almost only the parent compound (mouse liver contained small amounts of OH-

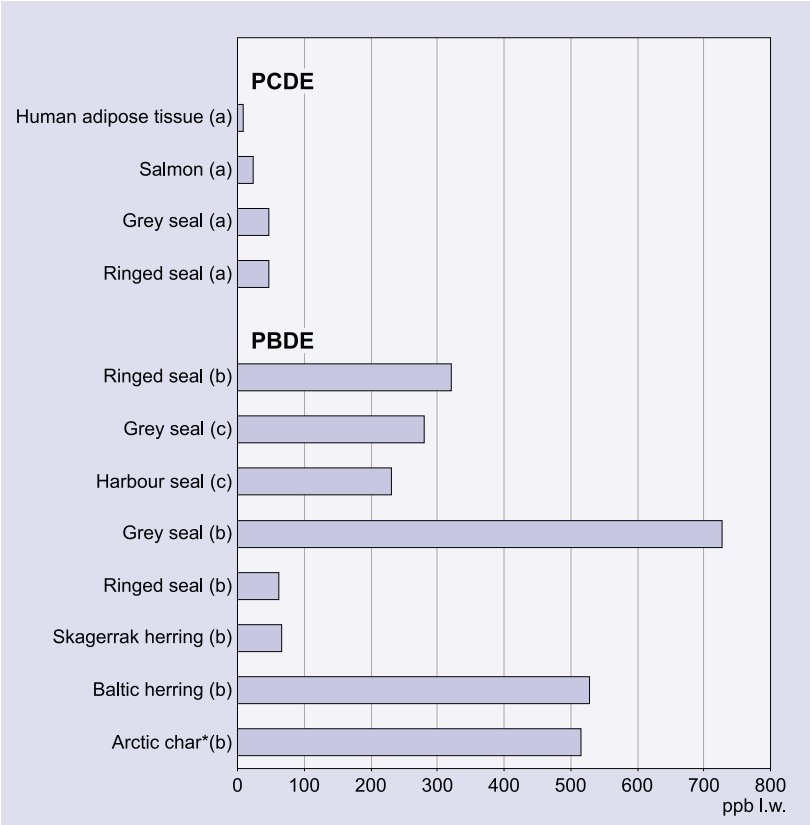


Figure 5.8. Levels of PBDE and PCDE in some biological samples from the Baltic area. Data from a = Koistinen et al 1995a; b = Jansson et al., 1993; c = Sellström 1996. **Salvelinus alpinus.*

PBDE) while hydroxylated metabolites were present in plasma from both species (Örn and Klasson Wehler, 1998).

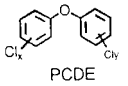
Environmental levels

Several PBDE congeners have been determined in the Swedish environment, and the 2,2',4,4'-tetraBDE is the most abundant congener (Sellström et al., 1993). Time trend studies for laminated sediment cores in the Baltic Sea show a 10-fold increase in concentration of 2,2',4,4'-tetraBDE and two penta BDE s from the latter half of the 1970s and 10 years forward (Nylund et al., 1992). The actual concentrations of individual PBDE congeners in the more recent sediment layers were only slightly lower than those of individual PCB congeners (eg., CB153; Nylund et al., 1992). In guillemot eggs from the Baltic area, an increase in PBDE concentration was also observed from 1970s to late 1980s. The trend during the last years is difficult to interpret since no obvious increase or decrease in concentration of PBDEs in guillemot eggs has been seen (Sellström et al., 1993). DecaBDE was not included in these analyses, nor in many others due to the analytical difficulties in determining this compound.

PBDEs have also been determined in eel from Dutch rivers and in cod liver from the North Sea with the highest concentration in the southern North Sea (de Boer 1989).

PBDEs have been determined in human adipose tissue from the national Human Adipose Tissue Survey repository from 1987 (USA). Hexa- to decaBDE were determined, generally in levels below 1 ppb (Stanley et al., 1991). The authors only had standards available for BDE congeners from hexa-decaBDE so tetra- and pentaBDEs were not determined. In plasma from Swedish male blood donors, 2,2',4,4'-tetraBDEs is present in 4 ppb l.w. (mean value of 10 samples, Klasson Wehler, et al., 1997).

Polychlorinated diphenyl ethers



PCDE

Polychlorinated diphenyl ethers (PCDEs) are byproducts in the production of chlorinated phenols. The byproducts may be present in concentrations up to 1000 ppm (Nilsson and Renberg, 1974). PCDEs have also been used for production of herbicides (Matsunaka, 1977). PCDEs have been determined in fly ash, probably formed during combustion (Kurz and Ballschmiter, 1995). The physical and chemical properties are similar to those of PBDEs, although PCDEs are not as sensitive to UV-light. Calculated log K_{OW} for decaCDE is 9.8 ±0.5 and for diCDE ca 5.4 ±0.3.

Several PCDEs have been determined at low ppb levels in the environment, e.g. in seals and salmon from the Baltic as well as in human adipose tissue (see below).

PCDEs are metabolized to phenolic metabolites at varying rates depending on structure and species. *Ortho*-hydroxylated PCDEs are also called pre-dioxins. An *ortho*-hydroxylated trichlorodiphenyl ether (2-OH-2',4,4'-triCDE) is used as an anti-microbial agent (Irgasan DP300) in fabrics and as a bacteriostat for shampoo, soaps and cosmetics (Hanioka et al., 1996).

Metabolism

Metabolism has been studied for several PCDE congeners in rats and in trout. The rat forms mainly *ortho*-substituted hydroxy-metabolites, at least of lower chlorinated congeners but scission of the ether bond also occurred as a minor metabolic pathway (Tulp et al., 1979). 2,2',4,5,5'-PentaCDE was excreted to 56%, primarily via faeces, by the rat within 7 days after an oral dose. The excreted material mainly consisted of unmetabolized pentaCDE. A plasma half-life of 5.8 days was calculated (Komsta et al., 1988). In trout, 4- mono- and 2,4-diCDE were primarily hydroxylated in the *para*-positions (Nilsson et al., 1978). The half-lives of 2,4,4'-tri-, 2,3',4,4'-tetra- and 2,2',4,4',5-pentaCDE in juvenile Atlantic salmon were 235 hr, 370 hr and 370 hr, respectively (Zitko and Carson, 1977).

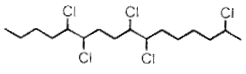
Environmental levels

PCDEs have been determined in Baltic species such as pike, salmon and grey and ringed seal at ppb levels (Figure 5.8, Koistinen et al., 1995a). The PCDE congener pattern varies but generally one penta-, 2–4 hexa-, and 2 heptaCDE congeners were present in all samples, that were close to pulp mills. In fish

(walleye (*Stizostedion vitreum*) and lake trout (*Salvelinus namaycush*)) from the Great Lakes, PCDE (tetra – decaCDE) concentrations were 126, 57, 16 and 4 ppb (total PCDE / wet weight), in whole fish from Lakes Ontairo, Erie, Huron and Superior, respectively (Niimi et al., 1994).

PCDE congeners, mainly a nonaCDE and decaCDE, have been determined in human adipose tissue samples from Finland, USA and Canada at levels in the low ppb range (Stanley et al., 1990 and 1991, Williams et al., 1991, Koistinen et al., 1995a). In the Finnish study, the congener pattern in human adipose tissue samples were compared with those in salmon and seal and were found to vary between the samples (Koistinen et al., 1995a,b). Many of the congeners found in the Finnish study were also found in cod liver oil samples where, in one of the two samples, the major congener (2,2',3,4,4',5,5'-heptaCDE) was present at a concentration similar to those of the most persistent PCB congeners (Kurz and Ballschmiter, 1995). By comparing the PCDE pattern in the cod liver samples with the congener patterns in fly ash, wood preservatives, and technical tetrachlorophenol, the source of the PCDE in the cod liver samples was suggested to be partly from chlorophenol-production but also from an unknown source (Kurz and Ballschmiter, 1995).

Chlorinated paraffins (CPs)



Chloroparaffin

Chlorinated paraffins are produced in large volumes for applications as plasticisers, flame retardants and the active component in cutting fluids. The world production of CPs in 1985 was 300000 tons and in Sweden, 900 tons were imported in 1995 (C_{10}-C_{17}) according to the Product register at the Swedish Chemical Inspectorate (Houghton 1993). The CPs consist of either short, intermediate or long chain polychlorinated alkane mixtures. The short chain CPs are C_{10}-C_{13}, intermediate chain lengths are C_{14}-C_{18}, and long chain CPs have $>C_{18}$. The CPs products have a variable chlorine content (40–70%) depending on the application. All CPs are extremely hydrophobic with log $K_{OW} > 6$. The number of isomers and homologues of CPs is high and includes many optically active compounds as well.

Metabolism and environmental levels

CPs are metabolised via oxidation to the corresponding carboxylic acids with the subsequent release of CO_2, a reaction that is inhibited at higher degree of chlorination (Darnerud et al., 1982). Sulfur-containing metabolites have also been indicated as degradation products of CPs (Åhlman et al., 1986).

CPs, even though difficult to analyse, have been determined in the environment at concentrations ranging from 130 ppb l.w. in Ringed seal to 1600 ppb l.w. in Skagerrak herring (Jansson et al., 1993). It is notable that high concentrations are observed in herring and terrestrial mammals whereas the levels are much lower in seals (Figure 5.9). CPs have also been determined in human tissues at 200 to 1500 ppb l.w. (Campbell and McConnel, 1980).

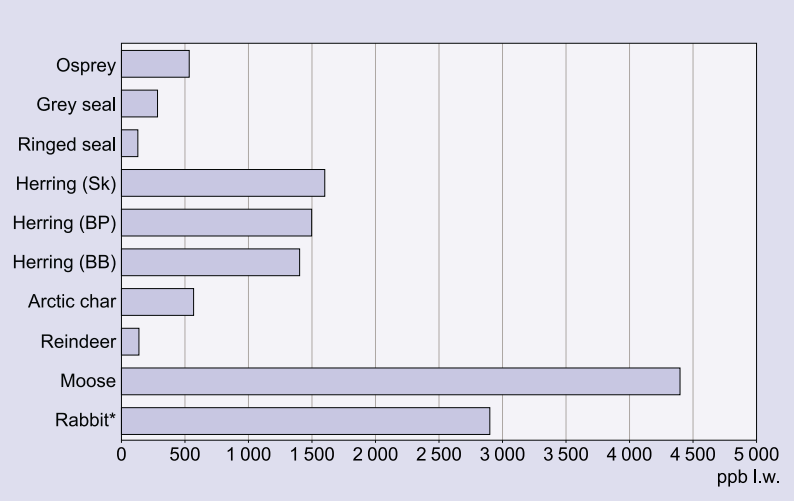
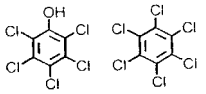


Figure 5.9. Levels of chlorinated paraffins (CPs) in some Swedish wildlife species (Jansson et al.,1993). Sk = Skagerrak; BP = Baltic Proper; BB = Bothnian bay.

Halogenated phenols and benzenes



PCP HCB

Halogenated phenols and benzenes are major industrial intermediates, solvents and some of these substances have been used as fungicides. Polyhalogenated benzenes, e.g. hexachlorobenzene (HCB), and polychlorinated and polybrominated, as well as mixed bromo-chloro phenols are also formed in incinerators (Tobin, 1986; Heeb et al., 1995). Brominated and mixed bromo-chloro phenols are registered as flame retardants and are also used as reagents in the production of other flame retardants. Chlorinated phenols are metabolic products of chlorinated benzenes (PCBz). Halogenated benzenes and phenols are increasingly lipophilic with increasing number of halogens, with the brominated analogues more lipophilic than the corresponding chlorinated compounds. Chlorinated phenols have log K_{OW} ranging from approximately 2 for monochlorophenols up to 5 for PCP (Shiu et al., 1994). The pK_a for phenols is decrease with increasing number of halogen substituents, and particularly number of *ortho*-substituted halogen atoms, e.g. PCP has a pK_a of 4.2 (Shui et al., 1994).

Halogenated benzenes are metabolized primarily to phenols, but also to methylthio ethers. HCB is thus metabolized to pentachlorophenol, which means that the presence of HCB in wildlife and humans must be considered also as an exposure to PCP (Figures 5.2, 5.3 and 5.4).

HCB is without question a major environmental contaminant of concern and further, PCP and phenols are of concern due to their presence in high concentrations in blood (See below).

Metabolism

Halogenated benzenes are metabolised to phenols as the major metabolites. Conjugation to sulphate and glucuronic acid increases the water-solubility and facilitates excretion. Metabolism via the mercapturic acid pathway also occurs, resulting in methyl sulphide metabolites (Koss et al., 1986). Thus, hexa- and pentachlorobenzene (HCBz, PeCBz) are both metabolized in the rat to pentachlorophenol (PCP) as the major metabolite (Koss et al., 1986; den Besten et al., 1993). PCP is excreted mainly via urine, and has been determined both as PCP and as conjugates in human urine (Koss et al., 1986). PCP is further metabolized, via reductive dechlorination, to lower chlorinated phenols, catechols and to tetrachlorohydroquinone (Renner and Hopfer, 1990, Koss et al., 1986, Ahlborg et al., 1978). The half-life of pentachlorophenol in rat plasma has been reported to be 5.6–9.5 h (Yuan et al., 1994) For humans, a urine elimination half-life of 17 days after a single oral dose has been reported (Uhl et al., 1986).

Environmental levels

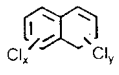
Hexachlorobenzene is a ubiquitous pollutant and has been determined in several species at different trophic levels in the Swedish environment (Figures 5.2 and 5.3). Pentachlorobenzene was determined in a few of those species but no chlorinated phenols could be determined, even though tri-to pentachlorophenols were included in the analysis (detection limit 3–140 ppb l.w.) (Jansson et al., 1993). The tissues analysed were muscle and/or adipose tissue which may explain the lack of chlorinated phenols, since these will be present primarily in blood due to their binding to proteins (van den Berg et al., 1991).

HCB is present in human adipose tissues at levels ranging from 10 ppb in a study from USA to 3800 ppb in a Greek study (Robinson et al., 1986). Human milk also contains HCB, but the levels in Sweden have dropped from 130 to 30 ppb l.w. during the last 20 years (Lundén and Norén, 1998).

PCP is the most studied of the phenols since it has been used in many applications and, in some cases of severe exposure even caused deaths to humans (Hattemeyer-Frey et al., 1989; Jorens and Schepens, 1993). PCP levels in human plasma of 15–75 ppb (f.w.) were reported for a control group in USA whereas a group of people living in log houses had 69–1340 ppb in their plasma (Cline et al., 1989). In this study, it was also noted that the children generally had 1.8 times higher concentrations than the parents, unrelated to the actual concentration in each case. An explanation was offered suggesting that the children's higher metabolic rate, i.e. higher frequency of breathing, increased their exposure. PCP has also been determined in human tissues with the highest concentration (l.w.) in testis (Wagner et al.,1991). PCP has been determined in human cerebrospinal fluid at levels 0.24–2 ppb (f.w.) in subjects having plasma concentrations of 4–60 ppb f.w. The concentration in cerebrospinal fluid was not correlated with the plasma levels in any of the persons studied (Jorens et al.,1991).

Chlorinated, brominated and mixed chlorobrominated phenols have been determined in human plasma (Eva Klasson Wehler, et al., 1997).

Polychlorinated naphthalenes (PCNs)



PCN

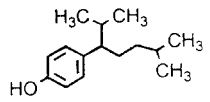
PCNs were, and may still be, used as dielectrical fluids and flame retardants in addition to be formed during combustion and in chlorine-alkali plants (Järnberg et al.,1993). PCNs are highly hydrophobic and have a coplanar structure. The persistence of PCN congeners in the environment varies with structure and metabolic capacity of different species (Asplund 1994).

PCNs are found in the environment at ppb levels (0.04–50 ppb l.w.) (Jansson et al., 1993). They are also present in human milk, total PCN was 0.48 ppb l.w. in 1992 (Figure 5.4). That is however almost a 10-fold decrease since 1972 (3.1 ppb l.w.) (Lundén and Norén, 1998).

Metabolism

Few metabolism studies on chlorinated naphthalenes have been performed, most of them with lower chlorinated PCN congeners. The metabolic rate depends on the structure and in an experiment with rats exposed to a technical mixture, it was shown that a few quantitatively minor components in the technical mixture (one pentaCN, two hexaCNs and one heptaCN) were selectively retained in the rat tissue, primarily in the liver, while the other congeners disappeared. These results were partly confirmed in a metabolism study in the rat, using a radiolabelled mixture of PCN. The oral dose was excreted, mainly as metabolites (hydroxy- and methylthio-metabolites), to 99% within 5 days. However, the metabolism occurs via highly reactive intermediates as shown by presence of covalently bound the material in the liver, kidney and lung (75, 67 and 59% of the total ^{14}C in the tissues) (Jakobsson 1994; Klasson Wehler unpublished). The dominating extractable compounds in the liver were the previously mentioned hexaCN congeners.

Alkyl phenols



p-Nonylphenol

Alkylphenols are primarily used after ethoxylation to alkylphenol ethoxylates (APE). These are non-ionic surfactants used as detergents, emulsifiers, wetting and dispersion agents in, e.g., paints. Alkylphenols are used as additives in lubricating oil, as spermicides in contraceptive foams and as antioxidants in PVC and polystyrene plastics (Platt, 1978). Nonylphenol (NP) has also been shown to be released from polycarbonate plastic products (Soto et al., 1991).

Alkylphenols are produced by alkylation of a phenol with an alkene yielding mainly *para*- but also *ortho*-substituted products. For technical productions, the alkyl-chain is branched and the degree of branching is determined by the alkene used for the coupling reaction. By a reaction with ethylene oxide, 2–40 ethoxy-groups are added to the phenol, forming alkyl phenol ethoxylates. The hydrophilicity of the APEs is determined by the number of ethoxylate-groups and the chain length of the alkyl group (Ahel and Giger, 1993).

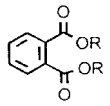
APEs are degraded by stepwise removal of the ethoxy-groups, yielding the parent alkylphenols, and concomitantly decreasing the hydrophilicity. The degradation rate of the APs depends mainly on the branching of the alkyl chain (Brunner et al., 1988; Ekelund et al., 1990). The log K_{OW} of e.g., 4-oktylphenol (OP) is 4.1 and for nonylphenol (NP) values vary from 4.2 to 6.4 (Shiu et al., 1994). NP and OP may thus be bioaccumulated and for NP, bioconcentration factors (BCFs) of 1,300 in fish and 3,400 in molluscs have been reported (Ekelund et al., 1990). With a decrease in alkyl chain lengths, the log K_{OW} decreases (Shiu et al., 1994). The pK_a of alkyl phenols range from 9 to 11 (Shiu et al., 1994).

Metabolism and environmental levels

Few metabolism studies of APs and/or APEs have been reported. In rats, nonoxynol-9 (NP with 9 ethoxy-groups) is metabolized to NP and subjected to glucuronidation. Radiolabelled nonoxynol-9 has been shown to be cleared from rat liver and kidneys within 48 h (Nimrod and Benson 1996). Degradation and metabolism studies have mainly been performed with straight chain alkyl whereas the technical products consist of mainly branched alkyl chains.

NP and NPE with 1–3 ethoxy-groups are detected in sewage systems effluents and NP has been determined in sediment near sources. Short-chain APs are also released from the deposits of oil-shale from Kothla-Järve in Estonia (personal communication, Åke Bergman). In Sweden, NP has been detected in recipients close to sources, such as downstream from a sewage treatment plant in Göta Älv (160 ppb in water) and at a concentration of 2 ppb in sea water in an industrially affected area at the Swedish west coast (Malmqvist and Duus, cited in TemaNord 1996). So far, reported data on environmental levels of APEs and APs are from industrially affected areas.

Phthalates



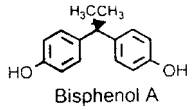
Phthalates

Phthalates are phthalic acid esterified with different alcohols, such as butanol, bensylalcohol and 2-ethylhexanol, and are used as plasticisers in many different applications. Dibutyl phthalate (DBP) and butylbenzyl phthalate (BBP) are included here due to reports on potential endocrine disrupting effects. DBP has a log K_{OW} between 4.6 and 4.9 and BBP between 4.9 and 5.2. Both are bioconcentrated in organisms at low trophic levels (mussels) but are metabolized and excreted by fish, birds and mammals, primarily as phthalic acid and the mono-ester (reviewed in TemaNORD 1996). Neither compound is considered to bioaccumulate in food chains and both are biodegradable.

DBP levels between 0.01 and 2 mg/l in river water in industrialized areas of Europe have been reported (Reviewed in TemaNORD 1996). Information is more limited on BBP but in 25% of samples from the Rhine (surface water and effluents) BBP was detected at a mean concentration of 0.078 mg/l (highest value 49 mg/l) (TemaNORD 1996).

Tetrabromophthalic anhydride is used as a flame retardant. In contact with water, this compound is hydrolysed to tetrabromophthalic acid.

Bisphenol A



Bisphenol A

Bisphenol A is used in the production of epoxy and polycarbonate resins, as a stabilizer in PVC and as an antioxidant in rubber and plastics. Bisphenol A is also active as a fungicide. In 1995, 18 tons were imported to Sweden as raw material for processes and 15 tons were imported included in chemical products (Prod. Reg. KemI). Log K_{OW} between 2.2 and 3.8 has been reported, and a water solubility of 120 mg/l at 25°C (TemaNORD 1996). The pK_a for bisphenol A is in the range of 9 to 11.

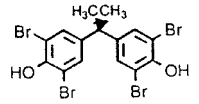
Only one study on the metabolism in mammals has been found, showing that bisphenol A is excreted by rats via faeces and urine, as such and/or as glucuronides (Knaak et al., 1966, cited in TemaNORD). The degradability of bisphenol A seems to be fairly rapid but not rapid enough to be defined as readily biodegradable (Tema NORD 1996).

There are almost no data on concentrations of bisphenol A in the environment. In a polluted river in the Tokyo area of Japan, 0.06–1.9 mg/l were determined but no data on bisphenol A levels in aquatic systems, sediments or terrestrial systems from Europe have been found. Bisphenol A has been reported to be released from lacquered cans (up to 33 mg/can), from polycarbonate flasks during autoclaving (up to 3.4 mg/l) and from dental cement (Krishnan et al., 1993; Brotons et al., 1994; Olea et al., 1996).

The high water solubility and fairly rapid biodegradation make it unlikely that bisphenol A should be bioaccumulated. At point sources and by contamination of food items, the compound may be a hazard.

Bisphenol B is structurally similar to bisphenol A except that one of the metyl groups is exchanged for an ethyl-group. The compound is used for production of phenolic resins.

Tetrabromobisphenol A



Tetrabromobisphenol A

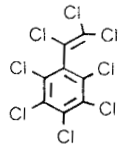
Tetrabromobisphenol A (TBBP-A) is used in the production epoxy-resins, for printed circuit boards, and ABS-polymers. TBBP-A is structurally similar to bisphenol A but has bromine atoms on the carbons *ortho* to the hydroxy-groups, which increases the hydrophobicity. The pK_a for TBBP-A will be much lower than for the non-brominated analogue and by comparing with the pK_a of OH-PCBs with different number of chloro-substituents (Ebner and Braselton, 1987), a pK_a lower than 7 can be expected.

There are no data available on metabolism or degradation of TBBP-A and only few studies on presence in the environment. In mussels and sediment in a polluted river in Japan, a concentration of 5 ppb wet weight mussel was determined. The concentration in the sediment was 20 ppb dry weight (Watanabe et al., 1983). In a Swedish study, TBBP-A was determined up- and downstream from a plastic industry producing epoxy-resins. The levels were ca 8 times higher downstream, indicating that the compound was released from the industry. TBBP-A was also determined in an electric circuit board showing that the polymerization is not quantitative (Sellström and Jansson, 1995).

TBBP-A has been shown to compete *in vitro* with thyroxine for the binding site on TTR (Brouwer, personal communication).

Additional compounds

For many compounds, data on potential adverse effects in general, and endocrine effects in particular, are very scarce and even absent in some cases. Compounds that are present in the environment are included in the general exposure. Two such compounds are therefore presented here as examples of compounds present in the environment.



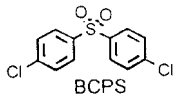
Octachlorostyrene

Octachlorostyrene (OCS) is an environmental contaminant with no known industrial application. It is formed as a byproduct in e.g. magnesium-production and in the chlorine-alkali process. Lower chlorinated styrene has been used in the flame retardant

industry (Kuryula and Papa, 1973). Log K_{OW} for OCS is 6.3–7.7, for tetraCS 4.3 and for diCS 3.4. OCS has been determined in fish and herons from the Great Lakes (10–430 ppb) (Kuehl et al.,1976; Reichel et al., 1977) and in plasma from occupationally exposed persons (up to 5 ppb) (Lunde and Bjorseth, 1977).

Metabolism

OCS is slowly metabolised and excreted. Only 8% of an iv dose was excreted, primarily as unchanged OCS, by the rat within 7 days. The highest concentrations (on a fresh weight basis) were found in adipose tissue and adrenals. Metabolism primarily took place on the vinyl-group but other metabolites were also formed (Chu et al., 1982). In cod exposed to radiolabelled OCS, high concentrations were observed in the brain (Ingebrigtsen et al., 1988). OCS has also been found in rain water in Ontario (1.6 ng/l) and in the river Elbe (1–1.9 ng/l) (Sanderson and Weis, 1989; Ernst et al., 1984).



BCPS

Bis(4-chlorophenyl) sulphone (BCPS): BCPS represents a new type of environmental contaminant, being a semivolatile plastic monomer (Brydson 1995). It is used for the production of polysulphones and amorphous polyarylene sulphides, but it is also used as a chemical in the textile dye industry and has been used as a pesticide in the former Soviet Union (Tarasenko 1969). It cannot be excluded that it is a by-product in other pesticides such as Tetradifon, Tetrasul, Sulphenone, and 4-chlorobenzene sulphone amides. There is little or no present use of BCPS in Sweden. BCPS is a hydrophobic compound with a calculated log K_{OW} of 4.1 and a chemical structure with resemblance to DDT. Commercial BCPS product contains minor amounts of two isomeric substances.

BCPS has been detected in biota in the Swedish and Latvian environments. The concentrations of BCPS in fish from 1994 and 1995 was on average 70 ppb l.w. which can be compared to the concentration of approximately 100 ppb l.w. of the persistent PCB congener CB138 (2,2',3,4,4'-5'-hexaCB) in the same sample. BCPS was also present in grey seal and white tailed sea eagle (Olsson and Bergman, 1995). BCPS has been detected in drinking water in USA (Lucas, 1984).

Estimation of human exposure

Human exposure to endocrine disrupting compounds may be expressed either as internal or external exposure. External exposure is the intake of compounds via food or inhalation whereas internal exposure is the presence of compounds in the body and therefore potential possibility to reach target tissues. The presence of pollutants in biota shows that external exposure for these compounds or their precursors has occurred. Internal exposure can be determined by analysis of levels in blood or tissues. Anthropogenic compounds present can via the blood reach all tissues and organs in the body. For steady-state situations, levels of most lipophilic persistent compounds in the blood are representative for concentrations in muscle or adipose tissue, when expressed on a lipid weight basis, and may therefore be used for estimation of body burden. This is not valid when selective tissue localization occurs due to binding of a compound to a specific macromolecule. Apart from this, a proper way to estimate the internal dose of exogenous compounds may thus be by analysis of blood plasma. Blood is particularly useful since the exposure to less persistent compounds, at least at the time of sampling, can also be determined. Another matrix for determining internal exposure is milk, which reflects the internal exposure of the mother. In addition, the milk is external exposure for the infant. An extensive data material has been obtained through analysis of mothers milk (e.g., Lundén and Norén, 1998; Norén et al., 1996). The concentrations of different pollutants in human milk are given in Figure 5.4.

External exposure is estimated by determining the concentrations of the pollutant in food items and estimating the dietary consumption. It should be noted that there may be large variations due to the difficulties in estimating diets and that any uptake via the lungs, which occasionally is an important route, e.g., for PCP, is not included (Cline et al., 1989). In most experimental toxicity studies it is only the external dose that is given and thus the daily intake is used for risk assessment. In Table 4.1, compounds that have been determined in human milk are listed and the estimated daily intake values are given.

In Denmark, human intake values for Dicofol, methoxychlor and HCHs have been estimated to be lower than 1.8 μg/day (TemaNord, 1996). In the UK, total daily intake of PCP was estimated to be 5.7 μg for the general population and 39 μg for an occupationally exposed population (34 μg due to inhalation) (Wild and Jones, 1992). In the UK, daily intake for phthalates was estimated to be 8 μg for BBP, 13 for DBP and 150 for DEHP. (MAFF, 1996). For the other compounds discussed in this chapter, PCN, halogenated phenols and benzenes other than HCB, AP, bisphenol-A, tetrabromobisphenol-A, PCN, PCDE, CPs, OCS and PCDE, there are no appropriate estimates available.

TABLE 5.1. Daily intake (μg/day) of xenobiotics from food, selected by their presence in mothers milk (cf Figure 4.4). Approximated averages for adults in Sweden.

Compound	Approximated daily intake (μg)	Reference
total PCB	3.2	Wicklund-Glynn et al, 1996
MSF-PCB	nd	
sDDT	1.2	Wicklund-Glynn pers. comm.
DDE	0.8	Wicklund-Glynn, pers. comm.
MSF-DDE	nd	
HCB	0.4	Wicklund-Glynn, pers. comm.
PCN	nd	
PDDD/F	<0.000147	De Wit et al 1997
α- and β-HCH	0.7	Wicklund-Glynn, pers. comm.
TCPM-OH	nd	
PBDE	0.2-0.7*	Darnerud, et al., 1998
PBB	nd	

nd = not determined;
* Rough estimate

SUMMARY

The chemical properties, environmental levels, metabolism, and use of several environmental contaminants have been described. The chemico-physical properties will determine the behaviour in the environment and in an organism. For some of the compounds (PCB, DDT, PCDD/F), there is much data whereas for some (TBBP-A, bisphenol-A, NP, BCPS), data on environmental levels, kinetics and metabolism are very limited.

PCBs and DDT are banned in Sweden but are still present at high concentrations in the environment. PCDE, HCB, OCS, TCPM and PCDE/Fs are byproducts and/or formed during, e.g., combustion and can therefore not be banned although emission can be regulated. PBDE, BCPS, CP, PBB, TBBP-A, NP and bisphenol-A are examples of compounds that are still being used. For PCN, the present use is unknown but the compound is found in products, and it is formed in certain industrial and combustion processes.

Compounds present in an organism provide evidence of exposure, and therefore levels of the compounds in wildlife and in humans have been presented. Persistent lipophilic compounds bioaccumulate in the organism and the internal exposure does thus increase.

Endocrine modulating effects may be caused by the parent compound but also after bioactivation due to metabolism. Metabolism may thus either produce or eliminate the active compound. The rate with which this occurs is crucial for potential effects. Therefore, data on kinetics and metabolism have been given when available. The structure of hydroxylated xenobiotics, and of hydroxylated xenobiotic metabolites, which have estrogenic effects are shown in Figure 5.10.

Some phenolic and methyl sulphonyl metabolites of environmental contaminants (PCB, DDT, HCB) are lipophilic and may be retained in organisms. Selective retention due to binding to proteins occurs in some cases.

Some compounds, such as halogenated diphenyl ethers and certain OH-PCBs, have an obvious structural similarity to thyroid hormones (Figure 5.11). Data on potential effects are however still lacking.

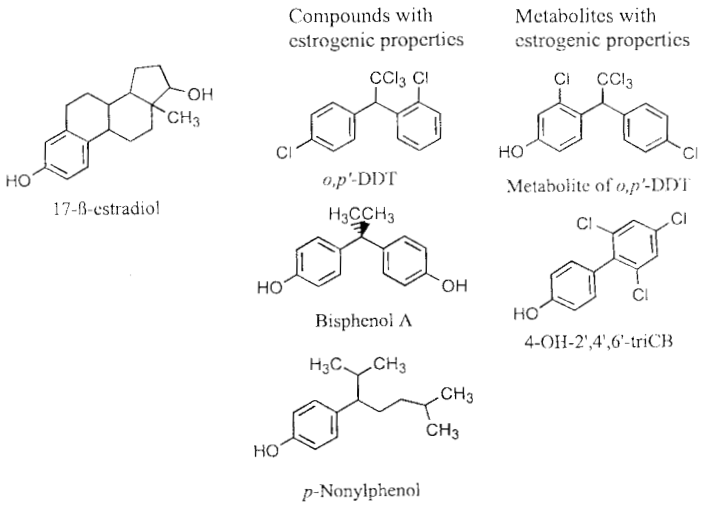


Figure 5.10. Structures of some xenobiotics and xenobiotic metabolites that have been reported to exert estrogenic effects.

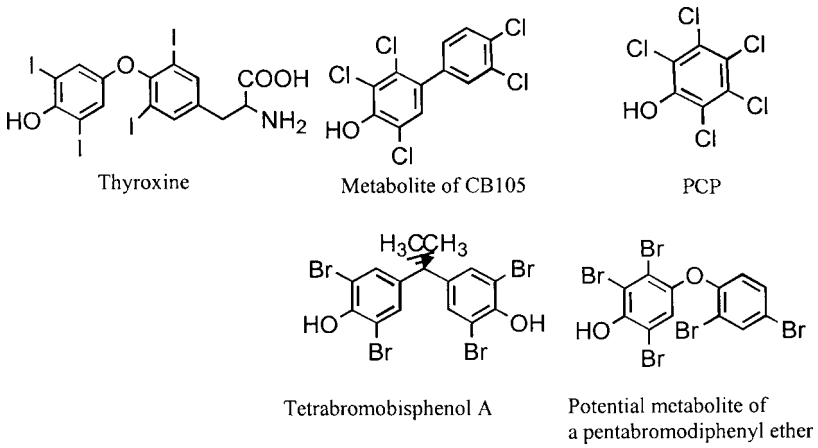


Figure 5.11. Structures of some phenolic, or metabolically hydroxylated, xenobiotics that compete with thyroxine for transthyretin, and structures of other potential competitors (to the right).

References

Ahel, M. & Giger, W. (1993) Partitioning of alkylphenols and alkylphenols polyethoxylates between water and organic solvents. Chemosphere 26:1471-1478.

Ahlborg, U.G., Larsson, K. And Thunberg, T. (1978) Metabolism of pentachlorophenol *in vivo* and i*n vitro*. Arch. Toxicol, 40:45-53.

Åhlman, M., Bergman, Å., Darnerud, P.O., Egestad, B. and Sjövall, J. (1986). Chlorinated Paraffins: Formation of Sulphur-containing metabolites of polychlorohexadecane in rats. Xenobiotica, 16:225-232.

Andersson, Ö., and Wartanian, A. (1992) Levels of polychlorinated camphenes (Toxaphene), chlordane compounds and polybrominated diphenyl ethers in seals from Swedish waters. Ambio, 21:550-552.

Aquilar, A., and Borrell, A. (1994) Reproductive transfer and variation of body load of organochlorine pollutants with age in Fin whales (*Balaenoptera physalus*). Arch. Environ. Contam. Toxicol, 27:546-554.

Ariyoshi, N., Oguri, K., Koga, N., Yoshimura, H. and Funae, Y. (1995) Metabolism of highly persistent PCB congener, 2,4,5,2',4',5'-hexachlorobiphenyl by human CYP2B6. Biochem. Biophys. Res. Comm. 212:455-460.

Asplund, L., Jansson, B., De Wit, C., Bergek, S., Hjelt, M., Rappe, C., Odsjö, T. and Olsson, M. (1990) Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) compared to other organohalogen pollutants in biological samples from Swedish ecosystems. Organohalogen compounds, Dioxin '90, EPRI seminar, 405-408.

Asplund, L. (1994) Development and application of methods for determination of polychlorinated organic pollutants in biota. Thesis, Stockholm University. ISBN 91-7153-272-2.

Asplund, L, Svensson, B.-G., Nilsson, A., Eriksson, U., Jansson, B., Jensen, S., Wideqvist, U. and Skerfving, S. (1994) PCB, *p,p'*-DDT and *p,p'*-DDE in human plasma related to fish consumption. Arch Environ. Health, 49:477-486.

Bakke, J.E., Bergman, Å.L. and Larsen, G.L. (1982) Metabolism of 2,4',5-trichlorobiphenyl in the mercapturic acid pathway. Science, 217:645-647.

Ballschmiter, K.H., Rappe, C. and Buser, H.R. (1989) Chemical properties, analytical methods and environmental levels of PCBs, PCTs, PCNs and PBBs. In: Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. (Eds. Kimbrough, R.D. and Jensen, A.A.). 2nd edition. Elsevier, Amsterdam, pp 47-69.

Ballschmitter, K., Mennel, A. and Buyten, J. (1993) Long chain alkyl-polysiloxanes as non-polar stationary phases in capillary gas chromatography. Fresenius J. Anal. Chem. 346: 396-402.

Becker, M., Phillips, T. and Safe, S. (1991) Polychlorinated diphenyl ethers – a review. Toxicol. Environ Chem. 33:189-200.

Bergek, S., Bergqvist, P.-A., Hjelt, M., Olsson, M., Rappe, Ch., Roos, A. and Zook, D. (1992) Concentrations of PCDDs and PCDFs in seals from Swedish waters. Ambio 21:553-556.

Bergman, Å., Norstrom, R., Kuroki, H. and Haraguchi, K. (1994b) PCB methyl sulphones in mammals from Canadian and Swedish environments. Environ. Toxicol. Chem., 13:121-128.

Bergman, Å. and Klasson Wehler, E. (1996) POP metabolites of toxicological/ecotoxicological concern. Third international symposium on Toxic Organic Compounds in the Environment. April 28 - May 3rd., Luhacovice, Czeck Republic.

Bergman, Å., Klasson Wehler, E. and Kuroki, H. (1994a) Selective retention of hydroxylated PCB metabolites in blood

from mammals. Environ. Health Perspec., 102:464-469.

Bergman, Å., Brandt, I. and Jansson, B. (1979) Accumulation of methylsulphonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. Toxicol. Appl. Pharmacol. 48:213-220.

Bignert, A., Litzén, K., Odsjö, T., Olsson, M., Persson, W. and Reutergårdh, L. (1995) Time-related factors influence the concentrations of sDDT, PCBs and shell parameters in eggs of Baltic guillemot (*Uria aalge*). Environ. Pollut. 89: 27-36.

Blomkvist, G., Roos, A., Jensen, S., Bignert, A. and Olsson, M. (1992) Concentrations of sDDT and PCB in seals from Swedish and Scottish waters. Ambio, 21:539-545.

Borlakoglu, J.T., and Haegele, K.D. (1991) Minireview: Comparative aspects on the bioaccumulation. Metabolism and toxicity eith PCBs. Comp. Biochem. Physiol. 100C:327-338.

Brandt, I. and Bergman, Å. (1987) PCB methyl sulphones and related compounds: Identification of target cells and tissues in different species. Chemosphere, 16: 1671-1676.

Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. (1994) Xenoestrogens relaesed from laquer coating in food cans. Environ. Health Perspec. 103:608-612.

Brunner, P.H., Capri, S., Marcomini, A. and Giger, W. (1988) Occurrence and behaviour of linear alkybenzenesulphonates, nonylphenol, nonylphenol mono- and nonylphneol diethoxylates in sewage and sewage sludge treatment. Wat. Res. 22:1465-1472.

Brydson, J.A. (1995) Plastic materials, 6th edition.pp 577-583, Butterworth-Heinemann, Linacre House, Oxford, UK. ISBN 0 7506 1864 7.

Burgaz, S., Afkham, B.L. and Karakaya, A.E. (1994) Organochlorine pesticide contaminants in human adipose tissue collected in Ankara (Turkey) 1991-1992 Bull Environ. Contam Toxicol 53:501-508.

Burgaz, S., Afkham, B.L. and Karakaya, A.E. (1995) Organochlorine pesticide contaminants in human adipose tissue collected in Tebriz (Iran) Bull Environ. Contam Toxicol 54:546-553.

Buser, H.-R. (1995) DDT, a potential source of environmental tris(4-chlorophenyl) methane and tris(4-chlorophenyl) methanol. Environ. Sci. Technol 29:2133-2139.

Campbell, I. and McConnel, G. (1980) Chlorinated paraffins and the environment. 1. Environmental Occurence. Environ. Sci. Technol., 14:1209-1214.

Chu, I., Villeneuve, D.C., Secours, V., Benoit, F.M. and Viau, A. (1982) The tissue distribution, metabolism and excretion of octachlorostyrene in the rat. Drug Metab. Dispos. 10:632-635.

Cline, R.E., Hill, R.H., Phillips, D.L. and Needham, L.L. (1989) Pentachlorophenol measurements in body fluids of people in log homes and work places. Arch. Environ. Contam. Toxicol, 18:475-481.

Darnerud, P.O., Biessman, A. and Brandt, I. (1982) Metabolic fate of chlorinated paraffins: Degree of chlorination of [^{14}C]-chlorodecanes in relation to degradation and excretion in mice. Arch Toxicol., 50:217-226.

Darnerud, P.O., Eriksen, G.S., Jóhanneson, T., Larsen, T.B., Viluksela, M. (1998) Polybrominated diphenyl ethers: food contamination and potential risks. Tema Nord, 1998:503. Nordic Council of Ministers.

De Boer, J. (1989) Organochlorine compounds and bromodiphenyl ethers in livers of atlantic cod (*Gadus morhua*) from the North Sea, 1977-1987. Chemosphere, 18:2131-2140.

De Boer, J., Wester, P.G., Everes, E.H.G. and Brinkman, U.A.Th. (1994) Determination of tris(4-chlorophenyl)methanol in fish, marine mammals and sediment. Environ. Pollut. 93:39-47.

De Wit, C., Järnberg, U., Asplund, L., Jansson, B., Olsson, M., Odsjö, T., Lindstedt, I., Andersson, Ö., Bergek, S., Hjelt, M., Rappe, C., Jansson, A. and Nygren, M. (1994) The Swedish dioxin survey: Summary of results from PCDD/F and coplanar PCB analyses in biota. Organohalogen Compounds 20:47-50.

Den Besten, C., Benni, M.H.J., Bruggeman, I., Schielen, P., Kuper, F., Brouwer, A., Koeman, J.H., Vos, J.G. and van Bladeren, P.J. (1993) The role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis: A comparison with pentachlorobenzene in a 13-week feeding study. Toxicol Appl. Pharmacol. 119: 181-194.

Ebner, K.V. and Braselton, Jr W.E. (1987) Structural and chemical requirements for hydroxychlorobiphenyls to uncouple rat liver mitochondira and potentiation of uncoupling with Aroclor 1254. Chem. Biol. Interact. 63:139-155.

Ekelund, R., Bergman, Å., Granmo, Å., and Berggren, M. (1990) Bioacumulation of 4-nonylphenol in Marine animals- a re-evaluation. Environ. Poll. 64:107-120.

El Dareer, S.M., Kalin, J.R., Tillery, K.F. and Hill, D.L. (1987) Disposition of decabromodiphenyl ether in rats dosed intravenously or by feeding. J. Toxicol. Environ. Health, 22:405-415.

Ernst, W., Weigelt, V. & Weber, K. (1984) Octachlorostyrene – a permanent micropollutant in the North Sea. Chemosphere 13:161.

Götz, R., Engo, P., Friesel, P., Roch, K., Kjeller, L.-O., Kulp, S.E., and Rappe, C. (1994) Sampling and analysis of water and suspended particulate matter of the river Elbe for polychlorinated dibenzo-*p*-dioxins (PCDDS) and dibenzofurans (PCDFS). Chemosphere 28:63-74.

Hanioka, N., Omae, E., Nishimura, T., Jinno, H., Onodera, S., Yoda, R. and Nado, M. (1996) Interaction of 2,4,4'-trichloro-2'-hydroxydiphenyl ether with microsomal cytochrome P450-dependent monooxygenases in rat liver. Chemosphere 33:265-276.

Haraguchi, K., Athanasiadou, M., Bergman, Å., Hovander, L. and Jensen, S. (1992) PCB and PCB methyl sulphones in selected groups of seals from the Swedish coastwaters. Ambio, 21:546-549.

Hattemeyer-Frey, H.A. and Trais, C.C. (1989) Pentachlorophenol: Environmental partitioning and human exposure. Arch. Environ. Contam. Toxicol. 18:482-489.

Heeb, N.V., Dolezal, I.S., Bührer, T., Mattrel, P., Wolfensberger, M. (1995) Distribution of halogenated phenols including mixed brominated and chlorinated phenols in municipal waste incineration flue gas. Chemosphere 31: 3033-3041.

Houghton, K.L. (1993). Chlorinated paraffins. In: the encyclopedia of chemical technology. Vol 6:78-87.

Ingebrigtsen, K., Solbakken, J.E., Norheim,G. and Nafstad, I. (1988). Distribution and elimination of [^{14}C] octachlorostyrene in cod (*Gadus morhua*), rainbow trout (*Salmo gairdneri*) and blue mussel (*Mytilus edulis*). J. Toxicol. Environ. Health, 25:361-72.

Jakobsson, Eva. (1994) Synthesis and analysis of chlorinated naphthalenes. Biological and environmental implications. Thesis, Stockholm University. ISBN 91 7153 241 2.

Jansen, H.T., Cooke, P.S., Porcelli, J., Liu, T.-C. and Hansen, L. (1993) Estrogenic and antiestrogenic actions of PCBs in the female rat: *In vitro* and *in vivo* studies. Reprod. Toxicol., 7:237-248.

Jansson, B., Jensen, S., Olsson, M., Renberg, L., Sundström, G., Vaz, R. (1975) Identification by GC-MS of phenolic metabolites of PCB and *p,p*'-DDE isolated from Baltic Guillemot and seal. Ambio 4:93-97.

Jansson, B., Andersson, R., Asplund, L., Litzén. K., Nylund, K., Sellström, U.,

Uvemo, U.-B., Wahlberg, C., Wideqvist, U., Odsjö, T. and Olsson, M. (1993) Chlorinated and brominated persistent organic compounds in biological samples from the environment. Environ. Toxicol. Chem., 12:1163-1174.

Jarman, W.M., Simon, M., Nostrom, R., Burns, S., Bacon, C.A., Simoneit, R.T. and Risebrough, R.W. (1992) Global distribution of tris(4-chlorophenyl) methanol in high trophic level birds and mammals. Environ. Sci. Technol. 26:1770-1774.

Jekat, F.W., Meisel, M.L., Eckard, R., Winterhoff, H. (1994) Effects of pentachlorophenol (PCP) on the pituitary and thyroidal hormone regulation in the rat. Toxicol Letters 71:9-25.

Johansen, R., Polder, A. and Utne Skaare, J. (1994) Congener-speicific determination of polychlorinated biphenyls and organochlorine pesticides in human milk from Norweigan mothers living in Oslo. J Toxicol. Environ. Health 42:157-171.

Jorens, P.G. and Schepens, P.J. (1993) Human pentachlorophenol poisoning. Human Experim. Toxicol 12:479-495.

Jorens, P.G., Janssens, J.J., van Tichelen, W.I., van Paesschen, W., de Deyn, P.P. and Schepens, P.J.C. (1991) Pentachlorophenol concentrations in human cerebrospinal fluid. Neurotoxicol. 12:1-8.

Järnberg, U., Asplund, L., de Wit, C., Grafström, A.-K., Haglund, P., Jansson, B., Lexén, K., Strandell, M., Olsson, M., and Jonsson, B. (1993) Polychlorinted biphenyls and naphthalenes in Swedish sediment and biota. Levels, patterns and time trends. Environ. Sci. Technol. 27: 1363-1374.

Kannan, N., Reusch, T., Schulz-Bull, D., Petrick, G. and Duincker, J. (1995) Chlorobiphenyls: Model compounds for metabolism in food chain organisms and their potential use as ecotoxicological stress indicators by application of the metabolic slope concept. Environ. Sci. Technol. 29:1851-1859.

Klasson Wehler, E., Bergman, Å., Darnerud, P.O. and Wachtmeister, C.A. (1989) 3,3',4,4'-Tetrachlorobiphenyl: Excretion and tissue retention of hydroxylated metabolites in the mouse. Drug. Metab. Dispos. 17:441-448.

Klasson Wehler, E., Hovander, L. and Lund, B.O. (1996) Comparative metabolism of 2,2',4,5,5'-pentachlorobiphenyl in mink (*Mustela vison*) and mouse. Chem. Res. Toxicol. 9:1340-1349.

Klasson Wehler, E., Lindberg, L., Jönsson, C.J. and Bergman Å. (1993) Tissue retention and metabolism of 2,3,3',4,4'-pentachlorobiphenyl in mink and mouse. Chemosphere, 27:2397-2412.

Klasson Wehler, E., Hovander, L. and Bergman, Å. (1997) New organohalogens in human plasma – Identification and quantification. Organohalogen Compunds 33:420-425. 17th International Meeting, Dioxin '97, Indianapolis, USA.

Koga, N., Kikuichi-Nishimura, N., Hara, T., Harada, N., Ishii, Y., Yamada, H., Oguri, K. and Yoshimura, H. (1995) Purification and characterization of a newly identified isoform of cytochrome P450 responsible for 3-hydroxylation of 2,5,2',5'-tetrachlorobiphenyl in hamster liver. Arch. Biochem Biophys. 317: 464-470.

Koga, N., Shinyama, A., Ishida, C., Hanioka, N. and Yoshimura, H. (1992) A new metabolite of 2,3,3',4'-tetrachlorobiphenyl in rat faeces. Chem. Pharm. Bull. 40: 3338-3339.

Koistinen, J., Mussalo-Rauhamaa, H. And Paasivirta, J. (1995a) Polychlorinated diphenyl ethers, dibenzo-*p*-dioxins and dibenzofurans in finnish human tissues compared to environmental samples. Chemosphere 31:4259-4271.

Koistinen, J., Paasivirta, J., Suonperä, M. and Hyvärinen, H. (1995b) Contamination of pike and sediment from the Kymijoki river by PCDEs, PCDDs, and PCDFs: Contents and patterns compared to pike and sediment from the Bothnian

Bay and seals from Lake Saimaa. Environ. Sci. Technol. 29:2541-2547.

Komsta, E., Chu, I., Villeneuve, D., Benoit, M. and Murdoch, D. (1988) Tissue distribution metabolism and excretion of 2,2', 4,5,5'-pentachlorodiphenyl ether in the rat. Arch. Toxicol. 62:258-262.

Koss, Gg., Reuter, A. and Koransky, W. (1986) Excretion of metabolites of hexachlorobenzene in the rat and in man. In: Hexachlorobenzene: Proceedings of an international symposium. (Ed's. Morris, C.R and Cabral, J.R.P.) IARC Scientific publications no 77:261-266. Oxford university press, UK. ISBN: 92-832-1177-4.

Kozie, K.D. and Anderson, R.K. (1991) Productivity, diet and environmental contaminants in Bald Eagles nesting near the Wisconsin shoreline of Lake Superior. Arch. Environ. Contam. Toxicol. 20:41-48.

Kreuger, J. (1995) Pesticides in rainwater in Sweden. In: Pesticides in precipitation and surface water. Tema Nord 1995:558 Nordic Council of Ministers, Copenhagen 1994, ISBN: 92 9120 678 4.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L. and Feldman, D. (1993) Bisphenol-A: An oestrogenic substance is released from polycarbonate flasks during autoclaving. Encocrinol. 132:2279-2286.

Krüger, C. (1988) Polybrominated biphenyl and polybrominated diphenyl ethers – detection and quantification in selected foods. Munster, University of Munster (Thesis).

Krüger, C., Fürst, P. and Groebel, W. (1988). Nachweis und bestimmung von polybromierten Biphenylen in Frauenmilch. Deutsche lebensmittel-rundschau. 9:273-276.

Kuehl, D.W., Johnson, K.L., Butterworth, B.C., Leonard, E.N. and Veith, G.D. (1981) Quantification of octachlorostyrene and related compounds in Great Lakes fish by gas chromatography-mass spectrometry. J. Great Lakes res. 7: 330-335.

Kuryula, W.C. and Papa, A.J. (1973) Flame retardancy of polymeric materials. Volume 1. Marcel Dekker Inc. New York.

Kurz, J. And Ballschmiter, K. (1995) Isomer-specific determination of 79 polychlorinated diphenyl ethers (PCDE) in cod liver oil, chlorophenols and in a fly ash. Fresenius J. Anal. Chem 351:98-109.

Lans, M.C., Klasson Wehler, E., Willemsen, M., Meussen, E., Safe, S. and Brouwer, A. (1993) Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and – dibenzofurans with human transthyretin. Chem. Biol. Interact, 88:7-21.

Larsen, B., Turrio-Baldassarri, L., Nilsson, T., Iacovella, N., diDomenico, A., Montagnam, M. and Facchetti, S. (1994) Toxic PCB congeners and organochlorine pesticides in Italian human milk. Ecotoxicol. Environ. Safety 28:1-13.

Letcher, R., Norstrom, R., Bergman, Å. (1995) Geographical distribution and identification of methyl sulfone PCB and DDE metabolites in pooled polar bear (*Ursus maritimus*) adipose tissue from western hemisphere Arctic and Subarctic regions. Sci. Total Environ. 160/161: 409-420.

Lucas, S.V. (1984) GC/MS analysis of organics in drinking water concentrates and advanced waste treatment concentrates. EPA-report 600/1-84-020 A&C. 1.133-162. 3:50-54.

Lunde, G. and Bjorseth, A. (1977) Human blood samples as indicators of occupational exposure to persistent chlorinated hydrocarbons. Sci. Total Environ. 8:241-246.

Lundén, Å. and Norén, K. (1998) Polychlorinated naphthalenes and other organochlorine contaminants in Swedish human milk, 1972-1992. Arch. Environ Contam. Toxicol. 34:414-423.

Luotamo, M., Järvisalo, J. and Aitio, A. (1991) Assessment of exposure to polychlorinated biphenyls: Analysis of selected isomers in blood and adipose tissue. Environ. Res., 54:121-134.

MAFF (1996) Ministry of Agricultural Food and Fisheries. Phthalates in food, food surveillance information sheet no. 82; 1996.

Matsunaka, S. (1977) Diphenyl ethers. In: Herbicides (eds P.C. Kearney and D.D. Kaufman) Vol 2, Marcel Dekker, New York, NY, pp 709-739.

Newsome, W.H. and Davies, D. (1996) Determination of PCB metabolites in Canadian human milk. Chemosphere 33:559-565.

Niimi, A.J., Metcalfe, C.D. and Huestis, S.Y. (1994) Chlorinated diphenyl ethers in Great Lakes fish and their environmental implication. Environm. Toxicol. Chem. 13:1133-1138.

Nilsson, C.A. and Renberg, L. (1974) Further studies on impurities in chlorophenols. J. Chromatogr. 89:325-333.

Nilsson, C.A., Norström, Å., Andersson, K. and Rappe, C. (1978). In: Pentachlorophenol: chemistry, pharmacology, and environmental toxicology. (Rao, K.R., ed) Plenum Press, pp 313-324, New York.

Nimrod, A.C. and Benson, W.H. (1996) Environmental estrogenic effects of alkylphenol ethoxylates. Crit. Reviews Toxicol. 26:335-364.

Norén, K., Lundén, Å., Pettersson, E. and Bergman, Å. (1996) Methylsulphonyl metabolites of polychlorinated biphenyls (PCBs) and DDE in Swedish human milk, 1972-1992. Environ. Health Perspec. 104:766-773.

Norstrom, R., Simon, M., Muir, D. and Schweinburg, R.E. (1988) Organochlorine contaminants in Arctic marine food chains: identification, geographical distribution, and temporal trends in polar bears. Environ. Sci. Technol. 22: 1063-1071.

Nylund, K., Asplund, L., Jansson, B., Jonsson, P., Litzén, K. and Sellström, U. (1992) Analysis of some polyhalogenated organic pollutants in sediment and sewage sludge. Chemosphere 24:1721-1730.

Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A., and Sonnenschein, C. (1996) Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspec. 104: 298-305.

Olsson, A. and Bergman, Å. (1995) A new persistent contaminant detected in Baltic Wildlife: Bis(4-chlorophenyl) sulphone. Ambio, 24:119-123.

Phillips. D., Smith, A., Burse, V., Steele, G., Needham, L. and Hannon, W. (1989) Halflife of polychlorinated biphenyls in occupationally exposed workers. Arch. Environ. Health 44:351-354.

Pijnenburg, A.M.C.M., Everts, J.W., de Boer, J., Boon J.P. (1995) Polybrominated biphenyl and diphenylether flame retardants: analysis, toxicity and evironmental occurence. Reviews Environ. Contam. Toxicol. 141:1-26.

Platt, A.E. (1978) Styrene plastics. In: Encyclopedia of Chemical Technology, 3rd ed. Vol 26 (R.E. Kirk and D.F. Othmer, eds) John Wiley and Sons, New York, pp 801-847.

POP – Stabila Organiska Ämnen. Stort eller litet problem. (1996) Naturvårdsverket (Swedish EPA) Rapport 4563. (In Swedish)

Preston, B.D., Miller, J.A., and Miller, E.C. (1983) Non-arene oxide aromatic ring hydroxylation of 2,2',5,5'-tetrachlorobiphenyl as the major metabolic pathway catalyzed by phenobarbital-induced rat liver microsomes. Drug metab. Dispos. 8:197-204.

Rahman, M.S., Montarella, L., Johansson, B and Larsen, B (1993). Trace levels of tris(4-chlorophenyl)-methanol and – me-

thane in human milk. Chemosphere, 27: 1487-1497.

Rapaport, R.A. and Eisenreich, S.J. (1984). Chromatographic determination of octanol-water partition coefficients (K_{ow}'s) for 58 polychlorinated biphenyl congeners. Environ. Sci. Technol. 18: 163-170.

Rappe, C. (1994) Dioxin, patterns and source identification. Fresenius J. Anal Chem. 348:63-75.

Reichel, W.L., Prouty, R.M. and Gay, M.L. (1977). Identification of polychlorinated styrene compounds in heron tissues by gas-liquid chromatography-mass spectrometry. J. Assoc. Off. Anal. Chem. 60:60-62.

Renner, G. and Hopfer, C. (1990) Metabolic studies of pentachlorophenol (PCP) in rats Xenobiotica 20:573-582.

Robinson, P.E., Lewczynski, B.A., Kutz, F.W. and Remmers, J.C. (1986). An evaluation of hexachlorobenzene body-burden levels in the general population of the USA. In: Hexachlorobenzene: Proceedings of an international symposium. (Ed's Morris, C.R. and Cabral, J.R.P.), IARC Scientific publications no 77:183-192, Oxford university press, UK. ISBN: 92 832 1177 4.

Ryan, J., Lizotte, R., Sakuma, T. and Mori, B. (1985) Chlorinated dibenzo-*p*-dioxins, chlorinated dibenzofurans and pentachlorophenol in Canadian chicken and pork samples. J. Agric. Food Chem. 33:1021-1026.

Sanderson, M. and Weis, I.M. (1989) Concentration s of two organic contaminants in precipitation, soils and plants in the Essez region of Southern Ontario. Environ. Poll. 59:41-54.

Schoula, R., Hajlova, Bencko, V., Poustka, J., Holadová, K. and Vizek, V. (1996) Occurence of persistent organochlorine contaminants in human milk collected in several regions of Czech republic. Chemosphere, 33:1485-1494.

Sellström, U. and Jansson, B. (1995) Analysis of tetrabromobisphenol A in a product and environmental samples. Chemosphere 31:3085-3092.

Sellström, U., Jansson, B., Kierkegaard, A., de Wit, C., Odsjö, T. and Olsson, M. (1993) Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. Chemosphere, 26:1703-1718.

Sellström, U., (1996) Polybrominated diphenyl ethers in the Swedish environment. Licentiate thesis, Environmental Chemistry, Stockholm University, Sweden. ITM-rapport 1996:45.

Shiu, W.-Y., Ma, K.-C., Varhaníková, D. and Mackay, D. (1994). Chlorophenols and alkylphenols: A review and correlation of environmentally relevant properties and fate in an envaluative environment. Chemosphere 29:1155-1224.

Soto, A.M., Justicia, H., Wray, J.W. and Sonnenschein, C. (1991) *p*-Nonyl-phenol: An estrogenic xenobiotic released from "modified" polystyrene. Environ. Health Perspec. 92:167-173.

Stanley, J.S., Cramer, P.H., Thornburg, K.R., Remmers, J.C., Breen, J.J. and Schwemberger, J. (1991) Mass spectral confirmation of chlorinated and brominated diphenylethers in human adipose tissues. Chemosphere 23:1185-1195.

Stanley, J., Cramer, P.H., Ayling, R.E., Thornburg, K.R., Remmeres, J.C., Breen, J.J., Schwemberger, J. (1990) Determination of the prevalence of polychlorinated diphenyl ethers (PCDPES) in human adipose tissue samples. Chemosphere 20:981-985.

Tanabe, S., Falandysz, J., Higaki, T., Kannan, K., Tatsukawa, R. (1993) Polychlorinated biphenyl and organochlorine insectide residues in human adipose tissue in Poland. Environ. Pollut 79:45-49.

Tarasenko, I.M. (1969) Development and adoption of measures for combating

pests of grapevines, fruit and forest crops. Lesovod. Agrolesomelior. 19:132-139.

TemaNORD 1996:580, Chemical with estrogen-like effects. Nordic Council of ministers, Copenhagen 1996. ISBN 92-9120-918-X.

Tobin, P. (1986) Known and potential sources of hexachlorobenzene. In: Hexachlorobenzene: Proceedings of an international symposium. (Ed's C.R. Morris and J.R.P. Cabral), IARC Scientific publications no 77:3-12. Oxford university press, UK. ISBN: 92 832 1177 4.

Toppari, J., Larsen, J., Christiansen, P., Giwercman, A., Grandjean, P., Guillette Jr, L., Jegou, B., Jensen, T., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J., Meyer, O., Muller, J., Rajpert-De-Meyts, E., Scheike, T, Sharpe, R., Sumpter, J., Skakkebaek, N. (1996) Male reproductive health and environmental xenoestrogens. Environ. Health Perspec. 104 (suppl 4):741-803.

Tulp, M.T.M., Sundström, G., Martron, L.B.J.M. and Hutzinger, O. (1979) Metabolism of chlorodiphenyl ethers and Irgasan® DP300. Xenobiotica 9:65-77.

Uhl, S., Schmid, P., Schlatter, C. (1986) Pharmacokinetics of pentachlorophenol in man. Arch. toxicol. 58:182-186.

Van den Berg, M., Blank, F., Heeremans, C., Wagenaar, H. and Olie, K. (1987) Presence of polychlorinated dibenzi-*p*-dioxins and polychlorinated dibenzofurans in fish-eating birds and fish from the Netherlands. Arch. Environ. Contam. Toxicol. 16:149–158.

Van den Berg, K.J., van Raaij, J.A.G.M., Bragt, P.C. and Notten, W.R.F. (1991) Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. Arch. Toxicol. 65:15-19.

von Meyerinck, L., Hufnagel, B., Schmoldt, A. and Benthe, H.F., (1990) Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. Toxicology, 61:259-274.

Wagner, S.L., Durand, L.R., Inman, R.D., Kiigemagi, U. and Deinzer, M.L. (1991) Residues of pentachlorophenol and other chlorinated contaminants in human tissues: analysis by electron capture gas chromatography and electron capture negative ion mass spectrometry. Arch. Environ. Contam. Toxicol. 21:596-606.

Waliszewski, S.M., Sedas, V.T.P., Infanzon, R.M. and Rivera, J. (1995) determination of organochlorine pesticide residues in human adipose tissue: 1992 study in Mexico. Bull. Environ. Contam. Toxicol. 55:43-49.

Walker, W., Risebrough, R.W., Jarman, W.M., de Lappe, B.W. and Tefft, J.A. (1989) Identification of tris(chlorophenyl)methanol in blubber from harbour seals from Puget Sound. Chemosphere, 18:1799-1804.

Watanabe, I., Kashimoto, T. and Tatsukawa, R. (1983) Identification of the flame-retardent tetrabromobisphenol A in the river sediment and the mussel collected in Osaka. Bull. Environ. Contam. Toxicol. 31:48-52.

Watanabe, I. and Tatsukawa, R. (1987) Formation of brominated dibenzofurans from the photolysis of flame retardant decabromodiphenyl ether in hexane solution by UV and sun light. Bull. Environ. Contam. Toxicol. 39:953-959.

Wicklund-Glynn, A., Darnerud, P.O., Andersson, Ö., Atuma, S., Johnsson, H., Linder, C-E-, Becker, W. (1996) Revised fish consumption advisory regarding PCBs and Dioxins, National Food Administration, Report 4/96.

Wild, S. and Jones, K.C. (1992) Pentachlorophenol in the UK environment II: Human exposure and an assessment of pathways. Chemosphere, 24:847-855.

Williams, D.T., Kennedy, B. and LeBel, G.L. (1991) Chlorinated diphenyl ethers

in human adipose tissue, part 2. Chemosphere 23:601-608.

Yoshimura, H., Yonemoto, Y., Yamada, H., Koga, N., Oguri, K., and Saeki, S. (1987) Metabolism *in vivo* of 3,3',4,4'-tetrachlorobiphenyl and toxicological assessments of the metabolites in rats. Xenobiotica, 17:897-910.

Yuan, J.H., Goehl, T.J., Murrill, E., Moore, R., Clark, K,. Hong, H.L. and Irwin, R.D. (1994) Toxicokinetics of pentachlorophenol in the F344 rat. Gavage and dosed feed studies. Xenobiotica, 24:553-560.

Zitko, V. and Carson, R. (1977) Uptake and excretion of chlorinated diphenyl ethers and brominated toluenes by fish. Chemosphere 6:293-301.

Zook, D., Buser, H.-R., Bergqvist, P.-A., Rappe, Ch. and Olsson, M. (1992) Detection of tris(chlorophenyl) methane and tris(4-chlorophenyl)methanol in Ringed seal (*Phoca hispida*) from the Baltic Sea. Ambio, 21:557-560.

Öberg, L,. Andersson, R. and Rappe, C. (1992) *De novo* formation of hepta- and octachlorodibenzo-*p*-dioxins from pentachlorophenol in municipal sewage sludge. Organohalogen compounds 9: 351-354.

Öberg, L., Wågman, N., Andersson, R. and Rappe, C. (1993) *De novo* formation of PCDD/Fs in compost and sewage sludge – a status report. Organohalogen Compounds 11:297-302.

Öberg, T. (1992) Bildningen av dioxin - litteraturstudier och utvärdering av mätdata. Jernkontoret 1991-10-25. (TAB - 92/1).

Öberg, L., Glas, B., Swanson, S., Rappe, C. and Paul, K. (1990) Peroxidase-catalyzed oxidation of chlorophenols to polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Arch. Environ. Contam. Toxicol. 19:930-938.

Örn, U. and Klasson Wehler, E (1998) Metabolism of 2,2',4,4'-tetrabromodiphenyl ether in rat and mouse. Xenobiotica, 28:199-211.

6. EXPERIMENTAL STUDIES OF DIFFERENT ENDOCRINE MODULATORS

PHYTOESTROGENS AND MYCOESTROGENS

Besides xenobiotics having endocrine effects, there are naturally occurring estrogenic compounds that are derived from plants (phytoestrogens) or fungi (mycoestrogens). These include, e.g., isoflavones (genistein and daidzen), coumestrol, and the mycotoxin zearalenone. While the synthetic environmental xenoestrogens are usually fairly persistent, the phytoestrogens are readily metabolized by most animals.

Reproductive and developmental disturbances

The reproductive toxicity of phytoestrogens and mycoestrogens was first recognized in livestock, and sheep and swine seem to be especially sensitive. In rams that were fed clover, which contains isoflavones, reduced sperm counts were noted (Kaldas and Hughes 1989) and ewes that graze clover rich in estrogens exhibit a syndrome characterized by decreased sexual behavior, altered cervical mucous and epithelium, and decreased fertility (Adams 1983). In swine, severe reproductive disorders have been observed after feeding with zearalenone-contaminated food. The disorders include infertility, abortion and vulvovaginalis (Mirocha et al., 1971). Inhibited reproduction in California quail (*Lophortyx californicus*) in a dry year was suggested to be due to ingestion of phytoestrogens which under dry conditions are produced in the leaves of stunted desert annuals (Leopold et al., 1976).

Interactions with hormones and hormone receptors

Steroid hormones

Following uptake from the gut, the plant isoflavones and lignans are metabolized into hormone-like compounds that both bind to the estrogen receptor and are able to induce estrogenic responses (Setchell and Adlercreutz 1988). The phytoestrogens induce steroid hormone binding globulin (SHBG) in the liver and this has been suggested to reduce the availability of the endogenous steroid hormones for the cells (Adlercreutz et al., 1995). This would result in reduced steroid hormone activity and to a reduction of their metabolism and clearance. The effects of phytoestrogens on the production of SHBG have been corroborated by *in vitro* studies using HepG2 cells (Mousavi and Adlercreutz 1993). As suggested by Adlercreutz and co-workers (1995), this may explain the high SHBG levels in vegetarians. In premenopausal women, dietary exposure to isoflavones modified sex hormone status (Cassidy et al., 1994, Cassidy and Bingham 1995).

The interaction of phytoestrogens with various estrogen-regulated systems does not necessarily lead to deleterious effects. It has been proposed that phytoestrogens, by inducing SHBG, reduce the hormone-related cancer incidence (Adlercreutz et al., 1995). It has for instance been shown that lignan-containing linseeds inhibit mammary tumor formation in rats (Serraino and Thompson 1991).

It has also been shown that phytoestrogens interact with the estrogen receptor and mediate estrogenic responses (Mathieson and Kitts 1980, Miksicek 1994, Scarlata and Miksicek 1995, Mellanen et al., 1996, Wang et al., 1996). In a study on ewes it was observed that genistein and coumestrol competed with estradiol-17β for the binding to the estrogen receptor (Mathieson and Kitt 1980). The binding affinity of several phyto- and mycoestrogens to the estrogen receptor has been determined *in vitro* assays (Miksicek 1994, Scarlata and Miksicek 1995). Zearalenone showed the highest binding affinity to the estrogen receptor. Other compounds that were found to bind in decreasing order of affinity were, β-zearalenol, coumestrol, genistein, diadzen, phloretin, fomononetin and biochanin A (Miksicek 1994). The binding of all these compounds was inhibited by 4-hydroxytamoxifen. Genistein has furthermore been shown to induce pS2 mRNA expression in MCF-7 cells at concentrations down to 10^{-8} M (Wang et al., 1996). In a study of wood-derived estrogenic substances it was shown that β-sitosterol and betulin conferred estrogenic responses (Mellanen et al., 1996). β-Sitosterol was found to increase proliferation in T-47D cells and to induce vitellogenin synthesis following injection into rainbow trout. Even though β-sitosterol binds poorly to the ER it is able to stimulate vitellogenin levels in male goldfish to 60% of the level attained with administering a similar dose of estrogen (Mac Latchy and van der Kraak 1995). However, the compound did not increase proliferation in MCF-7 cells. Betulin and abietic acid on the other hand activated proliferation in MCF-7 and T-47D cell lines, respectively, but did not induce vitellogenin in rainbow trout.

The recently identified new member of the estrogen receptor family (ER-β) has a higher relative binding affinity to coumestrol and genistein than ER-α while β-zearalenol binds equally well to both ER isoforms (Kuiper et al., 1997). Of the phytoestrogens tested, coumestrol was found to have the highest binding affinity to both ER isoforms, with a higher affinity than 17β-estradiol to ER-β. The two isoforms are differentially expressed in different rat tissues (Kuiper et al., 1997). Thus, the effect of phytoestrogens may differ between organs depending on the ER isoform that is most abundant as well as on the specific phytoestrogen to which the organism is exposed.

Intraperitoneal injection of β-sitosterol causes a decrease in plasma testosterone and 11-ketotestosterone in goldfish (MacLatchy and van der Kraak 1995). Isoflavones have been indicated to be potent inhibitors of β-hydroxysteroid dehydrogenase, an enzyme involved in steroid metabolism (Keung 1995). This enzyme is important in the conversion of pregnenolone to progesterone and testosterone.

Thyroid hormones

Lueprasitsakul et al., (1990) reported that a synthetic plant isoflavone displaced T4 from TTR and increased serum concentration of T4 in the rat. It has also been shown that soy protein increases T4 levels in the blood of laboratory animals (Forsythe 1995, Potter et al., 1996).

Retinoids

No data was found showing that phytoestrogens can modify retinoid pathways.

DDT

A number of studies have shown that DDT causes adverse effects on the reproduction of various animals. DDT compounds affect endocrine function in various ways and are known to interact both with the estrogen and androgen receptors. Although DDT exerts estrogenic effects in different animals, these effects may be mediated, at least in part, via inhibition of androgen function. *o,p'*-DDT is generally found to be the most potent estrogen receptor agonist of the DDT compounds, although it is orders of magnitude less potent than DES. It is probable that biotransformation of *o,p'*-DDT results in compounds being more potent estrogen receptor agonists than the parent compound. *p,p'*-DDE has proven to be very potent in reducing shell-thickness in various birds and it has recently been reported that this compound also acts as an antiandrogen. Another metabolite, 3-methylsulfonyl-DDE, has proven to be highly toxic to the glucocortioid-producing *zona fasciculata* in the adrenals.

Comprehensive criteria documents and risk assessments concerning DDT include IPCS (1979, 1989b) and US Dept. Health (1992).

Reproductive and developmental disturbances

That DDT can exert effects on sexual development was apparent already in the 50s when Burlington and Lindeman (1950) presented data indicating that injection of DDT into juvenile roosters resulted in reduced testicular size and female appearance. They suggested that DDT could have estrogenic activity.

Örberg et al., (1972) found that an intraperitoneal injection of DDT (40 mg/kg) in sexually mature female mice caused prolongation of the estrus cycle. When technical DDT (containing 15–20% *o,p'*-DDT) was administered orally (3 mg/kg body weight) to sexually mature female rabbits three times a week over a period of 12–15 weeks, a significantly reduced ovulation rate was observed (Lindenau et al., 1994).

Rat uterine ornithine decarboxylase activity is induced by estrogens and Bulger and Kupfer (1977) found that single i.p. injections of 0.5 mg of *o,p'*-DDT or 0.002 μg estradiol-17β stimulated the activity. The ED_{50} -values were 18 mg and 0.38 mg/kg body weight, respectively. The order of decreasing potency of DDT analogs was *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE.

In vivo uterotrophic action of o,p'-DDT in rats was inhibited by pretreatment with CCl_4 (Welch et al., 1969), indicating that cytochrome P450 was important for formation of active metabolites of the compound.

In birds, DDT compounds may affect embryonic development by reducing egg shell thickness or by direct effects on the embryos. Fry and Toone (1981) injected eggs of California gulls (*Larus californicus*) and western gulls (*Larus occidentalis*) with methoxychlor and *o,p'*- DDT and both compounds proved to cause feminization of male embryos. The feminized embryos exhibited left, or left and right oviducts and a left ovotestis. The most sensitive indicator of feminization was the presence of germ cells in a thickened ovary-like cortex in the left testis. Persistence of the right oviduct in female embryos was also ob-

served. In another study, injection of Japanese quail eggs with o,p´-DDT *in ovo* was found to have long-term effects and resulted in altered mating behavior in both males and females (Bryan et al., 1989).

In a two-generation study in American kestrels (*Falco sparverius*), paired females were exposed to the pesticide dicofol (MacLellan et al., 1996). Structurally, dicofol (1,1-*bis*(*p*-chlorophenyl)-2,2,2-trichloroethanol) is DDT with the addition of a hydroxy group. Females dosed with 20 mg/kg laid eggs with thinner shells than those of control birds. Male embryos from females dosed with 5 and 20 mg/kg exhibited gross morphological changes with enlarged left testes. Feminization of male embryos was confirmed by the presence of primordial germ cells in the male gonad.

The mechanism for eggshell thinning by DDE has not been completely elucidated although a number of possibilities have been put forward. Lundholm (1994, 1995) recently suggested that the probable mechanism for p,p'-DDE-induced eggshell thinning in ducks is an inhibition of prostaglandin synthesis in the eggshell gland mucosa. Lundholm (1995) proposed that prostaglandin stimulates the transport of HCO_3^- from the shell gland mucosa to the lumen. Inhibition of prostaglandin synthesis would then inhibit HCO_3^- transport and thereby also Ca^{2+} transport.

Effects of DDT on fish reproduction has been reviewed by Kime (1995). Treatment of trout, *Salmo trutta*, and brook char, *Salvelinus fontinalis*, with DDT (0.5–3.4 mg/kg bw per week) for extended periods results in decreased offspring survival. Similarily has egg and fry mortality in salmonids in polluted waters been correlated with DDT. Treatment of tilapia, *Oreochromis mossambicus*, with 1 mg DDT/l water for 20 days, resulted in inhibition of the steroidogenic enzymes in both testis and in ovaries. DDT given in unspecified, but probably high, doses causes abortion in the live-bearing mosquitofish, *Gambusia affinis*.

Interaction with hormones and hormone receptors

Steroid hormones

Inhibition of the binding of ^{3}H-estradiol to rat uterus cytosol by different DDT-related compounds was studied by Nelson (1974). *o,p'*-DDT was the most potent inhibitor, being approximately 2000 times less potent than DES. *o,p'*-DDD, *o,p'*-DDE, and methoxychlor were at least 20 times less potent and *p,p'*-DDD and *p,p'*-DDE showed very low activity. There was a positive correlation between inhibition of binding *in vitro* and uterotropic activity in immature rats *in vivo*.

In another *in vitro* study, the binding affinities of DES, *o,p'*-DDT and methoxychlor to the estrogen receptor in MCF-7 cells were compared to that of estradiol. The relative binding affinities when assayed in 100% human serum were 70% for DES, 0.04% for *o,p'*-DDT and <0.004% for methoxychlor (vom Saal et al., 1995). In serum-free medium, the relative affinities of DES and *o,p*'-DDT were about three times lower. These authors also studied the territorial behavior of male mice in adulthood following *in utero* exposure. The relative potencies of DES, *o,p*'-DDT, and methoxychlor in terms of increase in urine marking were similar as for estrogen receptor binding in the MCF-7 cells. A DES dose as low as 0.02 µg/kg maternal body weight/day from days 11–17 resulted in significant behavioral effects, whereas a 1000 times higher dose of *o,p'*-DDT and methoxychlor caused similar effects (vom Saal et al., 1995). *o,p'*-DDD, *o,p'*-DDT, DDOH, and *o,p'*-DDE were recently shown to bind to an estrogen receptor prepared from the oviduct of the American alligator with potencies approximately 300-, 1000-, 1000-, and 5000-fold lower than that of estradiol (Vonier et al., 1996).

Recently, Kelce et al., (1995) found that *p,p'*-

DDE bound to the androgen receptor and inhibited its activation. They also showed that injection of *p,p'*-DDE into juvenile male rats resulted in delayed onset of puberty while injection in older rats resulted in reduced androgen-dependent seminal vesicle and prostate weight.

Thyroid hormones

When DDT was administered to pigeons at a dose of 3 mg/kg b.w./day, symptoms of hyperthyroidism were noted but higher doses (6 to 54 mg/kg/day) caused hypothyroidism (Jefferies et al., 1971).

Retinoids

Studies in the rat demonstrate that exposure to DDT decreases the accumulation of newly absorbed vitamin A and decreases hepatic levels of vitamin A (Phillips 1963, Tinsley 1969, Azais et al., 1987). Decreased hepatic vitamin A levels were observed in the offspring of DDT-exposed female rats, at doses which did not affect the dams (Phillips et al., 1971). Penned cormorants (*Phalacrocorax a. auritus*) exposed to DDT in the diet for 9 weeks showed a dose-related decrease in the levels of hepatic vitamin A (Greighus et al., 1973). Human studies demonstrated increased serum vitamin A levels following occupational exposure to DDT (Keil et al., 1972, Nhachi et al., 1990).

LINDANE

Gamma-hexachlorocyclohexane (HCH) has the common name lindane and is the most toxic of the different HCH isomers (IPCS 1991). Lindane is an inducer of the hepatic MFO system and has been shown to interfere with steroid hormone metabolism. Lindane has been found to impair reproduction in experimental animals and the compound interferes with the hormone systems discussed in this report.

Reproductive and developmental effects

In female rabbits administered lindane (0.8 to 4 mg/kg b.w.), accumulation was high in the ovaries, oviducts, uterus and follicular fluid (Lindenau et al., 1994). A reduction in ovulation rate was observed following lindane administration. Exposure of male rats to 8 mg lindane/kg caused a decline in testicular weight and a cellular degeneration of Leydig cells (Chowdhury and Gautam 1994). When given orally to mice during early pregnancy (day 1–4), lindane caused absence of implantation sites (Sircar and Lahiri 1989). When given during day 6–12 of gestation it caused absorption of the fetuses, and when given late in pregnancy (days 14–19) lindane caused death of the pups following parturition. At birth the pups had reduced body weights. Administration of estrogen, but not progesterone, during early pregnancy counteracted the adverse effects of lindane on implantation. In another study, lindane was shown to delay vaginal opening and to disrupt the ovarian cyclicity in rats (Cooper et al., 1989). At doses of 20–40 mg/kg, lindane caused reduced pitutiary and uterine weights. Reduced levels of LH, estrogen, and prolactin and increased levels of FSH were observed following exposure to 40 mg lindane/kg (Cooper et al., 1989).

In a study in the domestic duck, lindane (20 mg/kg) caused egg shell thinning (Chakravarty and Lahiri 1986). Lindane has also been suggested to affect morphogenesis and func-

tionality of the thyroid gland during gland development in the clawed frog (Marchal-Seagault 1982).

Interactions with hormones and hormone receptors

Steroid hormones

Lindane does not by itself interact with the estrogen receptor (Laws et al., 1994, Flouriot et al., 1995). In rainbow trout hepatocytes, lindane exposure (100 μM) resulted in induction of vitellogenin and ER mRNA (Flouirot et al., 1995). However it was suggested that it was metabolites of lindane that were active. β-HCH produces estrogen-like effects such as stimulated proliferation in the human breast cancer cell lines MCF-7 and T47D. However, the action of β-HCH does not seem to be through the classic pathway of binding and activating the estrogen receptor (Steinmetz et al., 1996).

Lindane has been shown to reduce the serum testosterone levels in rats (Prasad et al., 1995) and catfish (*Clarias batrachus*) (Singh and Singh 1987). In rats it also reduced the epididymal sperm count and sperm mobility and increased the frequency of abnormal sperm (Prasad et al., 1995). In a study on rat liver microsomes it was observed that lindane induced 6α-, 7α- and 6β-hydroxylases (Haake et al., 1987).

Thyroid hormones

Disturbed function of the thyroid gland in the clawed frog *(Xenopus laevis*) was observed following lindane exposure (Marchal-Segault 1982). Lindane altered thyroid function in rats, resulting in increased T4 and reduced T3 levels (Seidler et al., 1976). In a study on catfish (*Heteropneustes fossilis*), it was observed that exposure to 8 ppm lindane resulted in reduced T4 levels while exposure to higher levels (16 ppm) increased the T4 levels in plasma (Yadav and Singh 1986). The T3 levels were reduced under these experimental conditions. It was suggested that lindane reduces the synthesis and release of thyroid hormones from the thyroid gland.

Retinoids

Comparable decreases in hepatic vitamin A levels were observed in hens exposed to oil solutions of DDT (2%), lindane (1%) and methoxychlor (5%) via gavage (1 ml) every other day for 15 weeks (Vavrova et al. 1976). No effect was observed after 5 weeks of treatment.

PCDDs/Fs

The most potent of the polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/Fs) is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD has been used as a model compound for other PCDDs/Fs and structurally related halogenated aromatic hydrocarbons that are agonists to the Ah receptor. Low doses of TCDD and related compounds cause developmental toxicity when administered at specific developmental stages. TCDD interacts with the hormone systems discussed in this report by altering hormone metabolism, interfering with hormone receptors, and modulating gene transcription. It is generally believed that the reproductive and developmental toxicity and other Ah receptor mediated effects of PCDDs/Fs are caused by the parent compounds and not by metabolites. Criteria documents include US/EPA (1985) and IPCS (1989a). The developmental and reproductive toxicity of dioxins and related compounds was reviewed by Peterson et al., (1993).

Reproductive and developmental disturbances

In a three-generation reproduction study, Sprague Dawley rats were exposed to 0, 0.001, 0.01, or 0.1 μg TCDD/kg b.w./day via the diet (Murray et al., 1979). The F_0 generation received the diet for 90 days before mating. Reduced litter size and decreased neonatal survival were observed in the F_0 generation at the 0.1 μg TCDD/kg dose. At a dose of 0.01 μg/kg/day, decreases in litter size at birth and in neonatal survival and growth were noted in the F_1 and F_2 but not in the F_0 generations. Peterson et al., (1993) compared the relationship between maternal toxicity and prenatal mortality in laboratory mammals exposed to TCDD during gestation. A general finding was that in nonprimate laboratory mammals, TCDD-induced prenatal mortality is most commonly associated with maternal toxicity that is not severe enough to result in maternal lethality.

A dose of 1 μg TCDD/kg/day administered 5 days a week for 13 weeks caused a decrease in spermatogenesis in the rat (Kociba et al., 1976). Single doses of 3.0 or 5.0 μg TCDD/kg to adult male rats caused a significant reduction in the number of spermatids/testis (Chahoud et al., 1992).

In the review by Peterson et al., (1993) it was concluded that developmental toxicity endpoints tend to be observed at lower TCDD exposure than are reproductive effects. When pregnant rats were treated with TCDD on day 15 of gestation, a decrease in androgenic status of the male offspring was found (Mably et al., 1992a). Ventral prostate weight was decreased by a maternal dose of 0.064 μg TCDD/kg and anogenital distance was decreased, testis descent was delayed, and seminal vesicle weight was decreased by a dose of 0.16 μg/kg. Plasma luteinizing hormone concentration was decreased by a lowest effective maternal dose of 1.0 μg/kg. At a maternal dose of 0.064 μg TCDD/kg, there were decreases in epididymis weight, cauda epididymis weight, sperm per cauda epididymis, daily sperm production rate and seminiferous tubule diameter whereas testis weight was decreased at a dose of 0.4 μg/kg. However, none of the doses (0.064–1.0 μg/kg) given to the mothers had any significant effect on the fertility of the males or on survival and growth of their offspring (Mably et al., 1992b). Less male-typical and more female-typical behavior was observed in male rats following *in utero* and lactational exposure to a maternal dose of 0.064–0.16 μg TCCD/kg (Mably et al., 1992c).

Cleft palate and hydronephrosis are structural malformations that have been observed in mice at doses of dioxin that do not cause either maternal or fetal toxicity. Hydronephrosis caused by dioxin is due to hyperplasia of the uretric epithelium, leading to blockage of the uretric lumen (Abbott et al., 1987). Treatment with T3 or T4 potentiates the induction of cleft palate by dioxin in the mouse (Lamb et al., 1986) and retinoic acid interacts synergistically with dioxin to induce cleft palate (Birnbaum et al., 1989; Abbott and Birnbaum, 1989) but has no effect on dioxin-induced hydronephrosis.

Gray et al., (1993; 1995) treated pregnant rats on day 15 of gestation with 1 mg TCDD/kg and examined both the male and the female offspring at birth, puberty, and adulthood. Results were generally similar to those previously reported by Mably et al., (1992). In the male offspring, a reduced anogenital distance was observed and puberty was delayed. Testicular sperm production was reduced and epididymal sperm counts and the number of sperm ejaculated were decreased. Ventral prostate and seminal vesicle weights were reduced. Subtle changes in male sexual behavior were also observed (Gray et al., 1995). In the female offspring, structural malformations in the urogenital tract were observed (Gray et al., 1993). Minimal to severe clefting of the phallus/clitoris was observed in the majority of the females, and severe clefting was accompanied by hypospadias. Vaginal open-

ing was incomplete and many of the pups had a persistent thread of tissue across the vaginal orifice.

That TCDD is highly embryotoxic in the chicken embryo was observed in egg-injection studies carried out by Higginbotham et al., (1968). An LD_{50} value for TCDD in chicken embryos was estimated to be 0.24 µg/kg egg weight (Allred and Strange 1977). Early life-stages of fish are sensitive to TCDD-induced mortality. In several species, TCDD has been found to cause an overt toxicity syndrome characterized by edema, hemorrhages, and arrested growth and development culminating in death (Peterson et al., 1993).

Interaction with hormones and hormone receptors

Steroid hormones

Serum testosterone and dihydrotestosterone were dose-dependently depressed in TCDD-treated rats, the ED_{50} being about 15 µg/kg body weight (Moore et al., 1985).

Dioxin does not bind to the estrogen receptor and does not cause classical estrogenic responses. TCDD inhibits several 17β-estradiol-induced responses in the rodent mammary and uterus and in human breast cancer cell lines (Safe and Krishnan, 1995). Although having antiestrogenic effects, dioxin does not block estrogen binding to the estrogen receptor. It rather appears that TCDD reduces the ability of the liganded estrogen receptor to bind to estrogen response elements, thereby reducing gene transcription (Kharat and Saatcioglu, 1996).

It has been observed that there are inhibitorydioxin-responsive elements in the estrogen regulated cathepsin-D gene (Krishnan et al., 1995, Safe and Krishnan 1995). Binding of TCDD-AhR to these sequences results in inhibition of estrogen dependent signaling. This is supported by the observation that in AhR negative cells TCDD is not antiestrogenic (Kharat and Saatcioglu 1996). The crosstalk between the AhR and ER systems is further indicated by the reduction in TCDD induced CYPIAI mRNA induction by exposure to estrogen.

Exposure of MCF-7 cells to 10 nM TCDD did not result in a significant reduction in ER mRNA levels (Gierthy et al., 1996). The specific binding of estradiol-17β to ER could be depressed by 49% under subsaturation conditions. This depression was reversed by the addition of α-naphthoflavone, an inhibitor of TCDD-induced metabolism of estradiol-17β. Thus, while TCDD does not affect the ER levels it may reduce the estrogenic response by increasing the metabolism of estradiol-17b.

In a ER-negative human breast cancer cell line, Hs578T, it has been observed that TCDD does not induce CYP1A1 induction (Wang et al., 1996). However, the inducibility of CYP1A1 could be reconstituted by transient transfection of this cell line with ER. It was also observed that only the N-terminal portion of the ER (amino acids 1–178) were needed to restore the TCDD responsiveness of the CYP1A1 gene promoter. In the human breast cancer cell line MDA–MB 231, which is also ER negative, it was found that TCDD did not induce CYP1A1 while other TCDD responsive genes, such as CYP1B1, were not affected (Dohr et al., 1995). From these studies it appears that ER is important for the specific upregulation of CYP1A1.

Even though TCDD does not bind to ER it has been observed that TCDD may in some instances result in activation of ER responsive genes. The partial agonistic effect of TCDD on ER responsive genes may be due to altered hormone metabolism.

Thyroid hormones

Several studies have shown that TCDD exposure of experimental animals affects plasma thyroid hormone levels. In rats TCDD decreases thyroxine (T4) levels already within the first 24 hours (Jones et al., 1987) and the degree and duration of reduction is more or less dose-related (Potter et

al., 1986, Gorski and Rozman 1987, Pohjanvirta et al., 1989). The effects on total T4 (TT4) and on the free fraction of T4 (FT4) in plasma are more or less similar (Potter et al., 1983, Gorski et al., 1988). However, the effects of TCDD on triiodothyronin (T3) levels are much more variable; in some studies, increases in plasma levels were observed, but decreases in T3 and unchanged levels have also been reported. The difference in direction of T3 changes could not easily be explained by TCDD level, time-point after administration or mode of administration, but will be discussed below in connection to proposed mechanisms.

It could be argued that some of the observed hormonal effects in experimental animals are secondary effects of the decrease in food intake, a well-known response to TCDD exposure. Indeed, studies on pair-fed groups of rats showed that the reduced food-intake did lower the T4 levels, but that this decrease was small in comparison to what was seen after TCDD exposure (Potter et al., 1986, Gorski et al., 1988).

Most experimental studies on thyroid hormone effects have been performed in rats. When other species were used, the decrease in T4 levels after TCDD exposure was less clear; instead, certain studies revealed an increase in T4 levels in plasma (guinea pigs: McKinney et al., 1985; hamsters: Henry and Gasiewicz 1987; mice: Birnbaum et al., 1989). Thus, species differences could complicate the interpretation of the data from experimental models.

The mechanism behind the TCDD-mediated modulation of thyroid hormones has been a matter of dispute, and several hypotheses have been presented. However, it has been suggest that the increased clearance of T4 by an enhanced biliary excretion of a conjugated T4 is the main mechanism behind the effects (Sewall et al., 1995). As stated by Sewall and coworkers (1995), the observed increase in UDP-GT1 mRNA, decrease in plasma levels of T4, increase in TSH, and thyroid hyperplasia resulting from chronic (30 d) TCDD exposure are consistent with effects emanating from a peripheral alteration of the pituitary-thyroid axis. The increased expression of UDP-GT1 mRNA was a sensitive effect in that study, and UDP-GT is reported to be induced through an Ah receptor-mediated mechanism (Bock 1991). This enzyme shows a selective activity towards phenolic type substrates (Hook et al., 1975, Lucier et al., 1975), including T4.

This proposed T4 deactivation mechanism could also explain why TCDD-mediated effects on T3 plasma levels are contradictory and more difficult to interpret. In contrast to T4, where the biliary excretion is a result of glucuronidation, the excretion of T3 occurs mainly by sulfation (LoPresto and Nicoloff 1994), which may not be induced by Ah receptor agonists. Another factor that may contribute to the maintenance of T3 levels is that TCDD induces 5'-monodeiodinase, which converts T4 to T3 (Potter et al., 1986).

Other mechanisms discussed to explain effects on thyroid hormone systems are related to the binding of TCDD to receptors and transport proteins. First, hydroxylated metabolites of PCDDs, but also of PCDFs and PCBs, bind to TTR *in vitro*, which suggests that the *in vivo* transport of T4 could be affected upon exposure to the parent compound (Lans et al., 1993). However, in man TTR is quantitatively not so important for plasma T4 transport, and thyroxine binding globulin (TBG) is instead the major T4 transporter. TBG does not bind the hydroxylated metabolites of the organochlorine compounds discussed above (Lans et al., 1994), which suggests that this mechanism is not important in man. Second, another mechanism discussed for thyroid hormone effects includes the observed alterations in levels of the nuclear thyroid hormone (T3) receptors, an effect depending on Ah-receptor binding and therefore seen after TCDD exposure of C57BL/6 but not DBA/2 mice (Bombick et al., 1988). Third, based on similarities in molecular structure between TCDD and

thyroid hormones (McKinney et al., 1985a), TCDD may act as a potent agonist for thyroxine; the binding to the nuclear receptor would consequently be affected. Finally, thyroid hormone effects could be a result of multiple hormonal interactions of the regulation and expression of genes ("cross-talk"). For example, it has been shown that glucocorticoid levels influence the binding capacity, as well as affinity, of the nuclear T3-binding site (Recupero et al., 1983).

Retinoids

Similarities in symptoms following dietary vitamin A deficiency and exposure to dioxin-like compounds have been noted by several authors (Kimbrough 1974, Innami et al., 1975, Thunberg et al., 1979, Zile 1992, Lu et al., 1994). The most prominent similarities include impaired growth, defective reproduction, developmental abnormalities, impaired immune function, and lesions of epithelial linings. Several studies have reported that TCDD treatment results in altered tissue retinoid levels and modulation of vitamin A metabolism (for review see Zile 1992). Reduction of hepatic retinoid levels is one of the most sensitive responses to TCDD exposure (VanBirgelen et al., 1994) and the response is also observed following *in utero* exposure (Moorse and Brouwer 1995). The decrease in hepatic retinoid levels is seen in all experimentally used rodent strains and species (Pohjanvirta et al., 1990, Håkansson et al., 1991). The potencies of individual PCDD and PCDF congeners to reduce hepatic vitamin A levels in the rat correlate well with their toxic potencies in subchronic (Håkansson et al., 1990) but not in single exposure studies (Ahlborg et al., 1987, 1989).

Reductions in retinoid levels, following TCDD exposure, have been reported in several extrahepatic tissues in most examined species (Brouwer et al., 1989, Håkansson et al., 1991), whereas increases in renal and serum retinoid levels have been repeatedly demonstrated only in the rat (Bank et al., 1989, Zile et al., 1989). In the TCDD-exposed rat, a dramatic and rapid increase in kidney retinoid content (Håkansson and Ahlborg 1985) is correlated, both on a time- and a dose-scale, to a sharp increase in renal LRAT-activity, and moderate increases in serum levels of both retinol and retinoic acid (Nilsson et al., 1996). Normally, renal vitamin A content is very low and increases only when liver vitamin A stores are nearing exhaustion, and serum vitamin A concentrations are normally tightly regulated within a narrow homeostatic range, which is thought to be determined by the release of retinol from the liver via regulation of vitamin A needs in extrahepatic tissues (Goodman 1984, Underwood 1984, Wolf 1984). Present data, thus, suggest that TCDD affects the proposed sensitive feed-back system between hepatic and renal vitamin A poolswhich are also linked to the pool of circulating vitamin A.

The possibility that TCDD could modulate gene transcription via the retinoic acid response element (RARE) is supported by the findings that TCDD and retinoic acid elicit a number of common biochemical and toxic responses both *in vivo* and *in vitro.* The intercactive effects of TCDD and retinoic acid are highly dependent on the cell or organ system under investigation. Berkers et al., (1994) reported a strong antagonistic effect of retinoic acid and several other retinoids on TCDD-induced terminal differentiation of primary cultured human keratinocytes. On the other hand, elicit a number of common biochemical and toxic responses both *in vivo* and *in vitro*.

The interactive effects of TCDD and TCDD and retinoic acid were reported to synergistically induce palatal clefts (Weston et al, 1995). Weston et al., (1995) demonstrated that TCDD is capable of inhibiting retinoic acid-induced expression of the CRABP-II and RAR genes in murine embryonic palate mesenchyme cells. Furthermore, retinoic acid and TCDD both inhibit mammary carcinogenesis in rodent models (NTP 1982, Moon et al.,

1992), and retinoic acid and TCDD elicit a number of common responses in MCF-7 cells, including inhibition of estrogen-induced cell proliferation, inhibition of nuclear estrogen receptor ligand binding and interactions with a consensus estrogen-responsive element (Lu et al., 1994). There are also data which demonstrate that TCDD induces the oxidation and conjugation of retinoids (Bank et al., 1989, Roberts et al., 1992)and that retinoic acid is able to induce the expression and activity of several dioxin-inducible enzymes (Matsuura and Ross 1993, Vecchini et al., 1995). Moreover, the presence of a retinoid responsive element was reported in the promoter region of the human CYP1A1 gene (Vecchini et al., 1994). The promoter region of CYP1A1 is known to also contain several dioxin responsive enhancer sequences (Denison et al., 1988, Whitlock 1993).

PCBs

From the results of many studies, of which some are presented here, it is clear that PCB causes impairment of reproduction and development in a number of animal species. At lower doses, reproductive effects have primarily been reported in nonhuman primates and mink. PCB may modulate endocrine function in various ways including, e.g., induction of hormone-metabolizing enzymes, interference with transport proteins, interaction with hormone receptors, and direct effects on endocrine organs. Certain effects, such as neurobehavioural changes, may appear in adulthood following exposure at sensitive periods during fetal and neonatal development. Metabolites of PCB may also have endocrine effects and it seems that hydroxylation is a prerequisite for chlorobiphenyls to act as estrogen receptor agonists. Hydroxylated congeners have been found to bind to the thyroid hormone-binding protein transthyretin (TTR), thereby interfering with the transport of thyroxine and retinol (vitamin A).

Due to the differences in their chlorination patterns, the various PCB congeners cause different biological effects. The effects of technical mixtures of PCB depend on the relative concentrations of the various congeners that they contain. The congeners lacking chlorine substituents in the *ortho* positions, but being chlorinated in the *para* positions and at least in one of the *meta* positions of each ring, are ligands with a high affinity for the Ah receptor. Thus these coplanar congeners exhibit biological effects similar to those caused by TCDD and other 2,3,7,8-substituted PCDDs/Fs. Like TCDD, the coplanar PCB congeners are antiestrogenic and disrupt retinoid storage processes. It seems that both the dioxin-like PCB congeners and those lacking affinity for the Ah receptor affect neurobehavioural development as well as thyroid function.

Examples of experimental studies on effects of PCB on reproduction, development and endocrine function are given below. In addition, there are several recent criteria documents and risk assessments concerning all aspects of PCB toxicity (US/EPA1990, IPCS1993, Nord 1992).

Reproductive and developmental disturbances

In mink, chronic exposure to 0.1 mg Clophen A50/animal (~kg)/day reduced kit survival and growth, an effect mainly attributed to the dioxin like congeners in the mixture (Brunström et al., 1991). At a three times higher dose, there was a reduced frequency of females whelping, and all kits born died within 24 h. Barsotti and Van Miller (1984) reported decreased birth weights in the offspring of rhesus monkeys (Macaca mulatta) receiving approximately 0.04 mg Aroclor 1016/kg b.w./day in a chronic study. However, the use of this study for regulatory purposes has been questioned (Kimbrough 1995). The developmental/reproductive toxicity of commercial PCB mixtures was reviewed by Golub et al., (1991). A LOAEL (lowest-observable-adverse-effect-level) for transient postnatal effects on growth and neurobehavioural development was identified at 0.25 mg Aroclor 1254/kg/day for rodents based on a study by Overmann et al., (1987). In rhesus monkeys (Macaca mulatta), subtle neurobehavioural effects were indicated in animals exposed perinatally and tested more than 3 years later (Levin et al., 1988). Maternal exposure was 0.03 mg Aroclor 1016/kg/day (1.0 ppm in diet) in that study, and this value was suggested by Golub et al., (1991) as a LOAEL for postnatal developmental end points.

Exposure of adult female mice to 0.025 mg Clophen A60/day for 75 days resulted in an increased length of the estrous cycle (Örberg and Kihlström 1973). In offspring of guinea-pigs exposed to a daily dose of 2.2 mg Clophen A50 during days 18 to 60 of gestation, vaginal opening was delayed in females and testis weight was decreased in males (Lundqvist 1990).

Following oral exposure of female rats to 16–64 mg Aroclor 1254 on days 1, 3, 5, 7, and 9 after birth, the sperm of their male offspring showed a reduced capacity to fertilize eggs (Sager and Girard 1994). No changes in production, morphology or motility of epididymal sperm were found in association with the reduced ability to fertilize eggs. Daily neonatal exposure (from day 1 to day 25) of rats to either Aroclor 1242 or 1254 resulted in increased adult testis weight and sperm production (Cooke et al., 1996). Aroclor 1254 was more potent, producing increases in testis weight of 13 and 23 % and in daily sperm production of 27 and 42 % at doses of 0.4 and 1.6 mg/day. It was suggested by the authors that PCBs produce these effects primarily by inducing hypothyroidism, which leads to increased Sertoli cell proliferation, testis weight, and daily sperm production. In a subchronic study, weanling (31-day-old) male rats were exposed to Aroclor 1254 (0.1–25 mg/kg and day for 5, 10 and 15 weeks) to examine effects on testicular gamete production and endocrine function (Gray, et al., 1993). Serum levels of thyroxine were depressed 30% in the lowest dose group (0.1 mg/kg) after 15 weeks and 25% in the 1 mg/kg dose group after 5 weeks. In the high dose group, body, seminal vesicle, cauda epididymal, and pituitary weights were depressed but no effects were observed on testes weights, testicular sperm numbers, sperm motility, and serum and testicular testosterone levels. Taken together these studies show that the developmental stage when the animals are exposed is important for the effects on male genitalia and they indicate that changes in thyroid hormone levels may be involved in the effects.

Increased uterine weight was observed following exposure of immature rats to Aroclor 1242, CB-52 (2,2',5,5'-tetraCB), and 4-OH-2',4',6'-PCB (80-640 mg for two consecutive days), indicating that these compounds are estrogenic (Jansen et al., 1993). CB-77 (160 µg, days 20 and 21) had no effect on uterine weight and attenuated the increase in uterine weight caused by either Aroclor 1242 or E2 treatment, suggesting that CB-77 is antiestrogenic.

The majority of publications on the developmental neurotoxicity of PCBs in rats and mon-

keys involve studies using commercial mixtures. In utero and lactational exposure of rats to Aroclor 1016 caused significant changes in biogenic amine (dopamine) levels in several brain regions (Seegal 1994). Perinatal exposure of rats to Aroclor 1254 resulted in significant alterations in synaptophysin and glial fibrillary acidic protein (GFAP) levels, i.e., neuronal and glial cell marker proteins, in several brain regions (Morse et al., 1996; Morse and Brouwer 1995). Prenatal, but not postnatal, PCB (Clophen A30) exposure (30 mg/kg in the diet) caused behavioral changes in rats (Lilienthal and Winneke 1991). In mice, neonatal exposure to both dioxin-like and non dioxin-like PCB congeners can induce permanent aberrations in expression of cholinergic receptors and spontaneous behavior of the adults (Eriksson et al., 1991, Eriksson and Fredriksson 1996). Thus, PCBs affect development of the nervous system via different mechanisms and effects induced perinatally may persist in adulthood.

In the chicken, egg hatchability is severely depressed at PCB levels which do not decrease egg production. Scott et al., (1975) found that hatching of chicken eggs was completely prevented at residue levels of about 5 ppm. Dioxin-like PCB congeners are highly embryotoxic in the chicken whereas embryos from a number of other avian species are less sensitive to these compounds (Brunström 1988).

Effects of PCBs on fish reproduction has been reviewed by Kime (1995). Administration of PCBs have in several studies impaired reproductive parameters. Administration of PCB to Atlantic croaker, *Micropogonias undulatus* (from 0.05 mg/kg body weight) or to the rainbow trout via the food (3–300 mg/kg diet for 180 days) impair vitellogenesis. Feeding of minnows, *Phoxinus phoxinus* (20–200 mg/kg diet) results in low hatchability in the eggs. Both ovarian and testes weights have been suppressed by PCB treatment in the Atlantic croaker. PCB administration (has also been found to suppress circulating levels of both estrogens and androgens in carp, *Cyprinus carpio* (25 mg/kg bw intraperitoneally), rainbow trout (25 mg/kg bw intraperitoneally) and Atlantic croaker (3–5 mg/kg bw). Damages on spermatogenesis, enzymes involved in steroidogenesis, pituitary gonadotropic secretion and oocyte development have also been observed in fish after PCB treatment.

Interactions with hormones and hormone receptors

Steroid hormones

The PCB mixtures Aroclor 1221 and 1254 inhibited binding of ^{3}H-estradiol to rat uterus cytosol *in vitro*, but they were 3–5 orders of magnitude less potent than DES (Nelson, 1974).

In pregnant mink, both CB-153 (2,2',4,4',5,5'-hexaCB, 20 mg/kg) and CB-169 (3,3',4,4',5,5'-hexaCB, 0.4 and 0.8 mg/kg) significantly increased uterine progesterone receptor dissociation constants (Patnode and Curtis 1994). CB-153 and the higher dose of CB-169 also caused increased uterine estrogen and progesterone receptor concentrations during gestation.

Korach et al., (1988) tested a series of polychlorinated hydroxybiphenyls for their binding to the estrogen receptor *in vitro*. Compounds with the stronger affinities contained single or multiple *ortho*-chlorine substitutions. Ortho-substitution creates a bulky group which was suggested to enhance the affinity for the ER. 4-OH-2',4',6'-PCB showed the strongest binding affinity of the tested compounds, having 42 times lower receptor affinity than estradiol. Both non- and mono-*ortho*-chlorinated PCB congeners were antiestrogenic in MCF-7 cells, inhibiting estrogen-induced secretion of procathepsin-D (Krishnan and Safe, 1993). CB-126 was the most potent congener causing antiestrogenic effects at concentrations (1 nM) 100 times lower than the mono-*ortho* PCBs (100 nM). Several Aroclor mixtures were not antiestro-

genic at the highest concentrations tested (1 mM).

The effects of a mixture of CB-61 (2,3,4,5-tetraCB), CB-101 (2,2',4,5,5'-pentaCB), CB-136 (2,2',3,3',6,6'-hexaCB), and CB-194 (2,2',3,3',4,4',5,5'-octaCB) on hCG-stimulated androgen production were studied in suspensions of Leydig cells from adult rat testis (Kovacevic et al., 1995). The PCB mixture significantly inhibited androgen (testosterone + dihydro-testosterone) production and it was suggested that the activity of the microsomal enzyme $P450_{SCC}$ (side-chain cleavage) was decreased by the PCB treatment.

Cultured anterior pituitary cells from female rats exposed to Aroclor 1242 *in vitro* exhibited enhanced LH and FSH release in response to gonadotropin-releasing hormone (Jansen et al., 1993). However, E2 was 4 to 5 orders of magnitude more potent than Aroclor 1242.

In a study on zebrafish injected with 1 mmol/kg of CB-60 (2,3,4,4'-tetraCB), CB-104 (2,2',4,6,6'-pentaCB), CB-173 (2,2', 3,3',,4,5,6-heptaCB), or CB-190 (2,3,3',4,4', 5,6-heptaCB), it was found that only CB-104 induced elevated estrogen receptor levels in the liver (Billsson and Olsson, unpublished).

Thyroid hormones

Certain OH-PCBs have been shown to compete with thyroxine for the binding site on TTR (Lans et al., 1993). The binding of 4-OH-3,3',4',5-tetraCB, which is a metabolite of CB-77 (Klasson Wehler et al., 1989), to transthyretin prevented the formation of the RBP-TTR complex that transports vitamin A from the liver to the peripheral tissues (Brouwer and van den Berg 1986). This OH-PCB is also known to be transported across the placenta in rats and mice and to accumulate in the fetuses (Darnerud et al., 1996; Morse et al., 1996). When rats were exposed to 5 or 25 mg Aroclor/kg/day on days 10–16 of gestation, fetal and neonatal plasma T4 levels were reduced (Morse et al., 1996). The concentration of T4 was markedly decreased in the fetal brain on day 20 of gestation whereas decreases in T3 levels were less dramatic. The effects were suggested to be caused by OH-PCBs accumulated in the fetuses (Morse et al., 1996). When Aroclor 1254 (62.5–250 ppm in the diet) was given to rats during gestation and lactation, depressed T_4, but not T_3, levels were observed in the pups (Juárez de Ku et al., 1994). Goldey et al., (1995) reported a dose-dependent reduction in plasma thyroxine levels in offspring of rats exposed to Aroclor 1254 (1–8 mg/kg bw) on gestation day 6 through postnatal day 21. By weaning, pup mortality was 20% in the 4 mg/kg group and 50% at the highest dose. Deficits in body weight gain, early eye opening, and hearing deficits were apparent in the surviving pups. Hypothyroidism occurring during auditory system development causes abnormalities of the cochlea, and the authors suggested that the auditory deficits reflected effects of the observed PCB-induced hypothyroidism on the development of the cochlea. Perinatal exposure to CB-77 or 2,3,7,8-TCDD (8 mg and 0.1 µg/kg/day on gestation days 10–16) significantly reduced (15–20%) plasma thyroxine levels in weanling rats (Seo et al., 1995). An induction of UDPGT was suggested to explain the reduction. In a similar study, rats were exposed to CB-28 (2,4,4'-triCB), 8 or 32 mg/kg/day; CB-118 (2,3',4,4',5-pentaCB), 4 or 16 mg/kg/day; or CB-153 (2,2',4,4',5,5'-hexaCB), 16 or 64 mg/kg/day (Ness et al., 1993). At weaning, serum T_4, but not T_3, was markedly depressed in the pups, but not in the dams, exposed to CB-118 and CB-153. Histological evaluation of thyroids from the CB-118-exposed pups showed changes suggestive of sustained TSH stimulation.

The mechanisms for the decreases in plasma thyroid hormone levels caused by PCBs are not clear but have been suggested to be due to interference with their plasma transport or induction of hepatic glucuronidation of thyroxine. Changes in thyroid hormone homeostasis

during fetal and postnatal life may cause severe developmental disturbances.

Retinoids

Many of the symptoms of PCB intoxication resemble those of vitamin A deficiency (for review see Zile 1992). As compared to PCDDs and PCDFs (see 6.8.4), PCBs comprise a more heterogenous group of chemical compounds. The more planar PCB congeners, which lack or have few substituents in the *ortho*-positions, modify retinoid levels and metabolism in a similar way to dioxins, although PCB congeners are less potent than the corresponding dioxin. The following relative potency values (compared to TCDD) for hepatic vitamin A reduction have been determined in rats, which were exposed for 13 weeks through their diet: 0.05 for CB-126 (3,3',4,4',5-pentaCB), 0.0005 for CB-156 (2,3,3',4,4', 5-hexaCB), 0.0002 for CB-105 (2,3,3',4,4'-pentaCB), 0.0001 for CB-77 and 0.00001 for CB-153 (Chu et al., 1994, 1995, 1996a, 1996c), whereas CB-128 (2,2',3,3',4,4'-hexaCB) had a potency of 0.00001, but only in female rats (Chu et al. 1996b). CB-118 (Chu et al., 1995) and CB-28 (2,4,4'-triCB) (Chu et al., 1996c) were unable to reduce hepatic vitamin A levels. After single exposure, CB-122 (2',3,3',4,5-pentaCB) caused decreased hepatic vitamin A levels in rodents comparable to the reductions caused by CB-77 (Chen et al., 1992).

Effects of PCBs on hepatic retinoid levels are seen in all experimentally used rodent strains (Innami et al., 1975, Håkansson et al., 1991, Chu et al., 1994, 1995, 1996a,b,c), primates (Allen 1975, Allen et al., 1978), mink (Brunström et al.,

1991) and quail (Bitman et al., 1972, Cecil et al., 1973). Morse and Brouwer (1995) reported on permanent changes in hepatic retinoid status in prenatally exposed rat offspring at a dosage level of Aroclor 1254 that did not induce overt toxicity.

In contrast to TCDD, which increases plasma retinoid levels (Bank et al., 1989, Van Birgelen et al., 1994, Nilsson et al., 1996), there are some PCB congeners which decrease plasma vitamin A levels (Brouwer et al., 1985, Azais et al., 1987, Chen et al., 1992). The plasma retinol reductions by CB-77 appear to be caused by binding of a hydroxy-metabolite to the thyroxine-binding site on transthyretin. Binding of the OH-metabolite inhibits the binding of the retinol-RBP complex to transthyretin, which results in an increased glomerular clearance of retinol bound-RBP (Brouwer and Van den Berg 1986, Brouwer et al., 1989).

Reductions in retinoid levels have also been reported in extrahepatic tissues, such as skin of marmoset monkeys (*Callithrix jacchus*) (Brouwer 1987), and lungs of rats (Håkansson et al., 1991,Chu et al., 1994, 1995, 1996a,b,c) and mink (Brunström et al., 1991).

There was no effect on tissue retinoid levels in mink chronically exposed to a mixture of 3-methylsulfonyl-DDE and 15 methylsulfonyl-PCBs (Lund et al., 1997).

PBDEs

Polybrominated diphenyl ethers (PBDEs) have been used in increasing amounts as flame retardants, but still the biological effects of this group of chemicals are not well known (KemI 1994). However, a recent comprehensive criteria document on PBDEs has been published by IPCS (1994b). Based on present studies two target tissues may be identified, the liver and the thyroid gland. Also, in some reproductive studies signs of fetal toxicity were evident.

Reproductive effects

One of the studies on reproduction toxicity of PBDEs is a reproductive performance study (decaBDE), whereas the other are teratogenicity studies (decaBDE, octaBDE, Saytex 111. i.e. commercial mixtures containing mainly hexa- (9%), hepta- (45%) and octa- (34%) BDE), and pentaBDE). All studies are performed on rats except the Saytex study (both rats and rabbits).

In the reproductive performance study on decaBDE, no compound-related effects were seen (IPCS 1994b). The teratogenicity study (0–1000 mg/kg, day 6–15) revealed no external abnormalities. However, decaBDE resulted in increased numbers of litters with subcutaneous edema and delayed ossification of bones of the skull (1000 mg/kg). The octaBDE increased the number of late resorptions, significantly reduced mean fetal weights, and resulted in edema and reduced ossification of various bones at the dose 50 mg/kg; day 6–15 (IPCS 1994b). At this dose, some signs on maternal toxicity were also evident (reduced body weight gain and increased cholesterol levels). The Saytex 111 exposure in rats resulted in some effects in the fetus (delayed ossification, enlarged heart, "rear limb malformation") at a dose (25 mg/kg) that was non-toxic to the dam. The Saytex 111-exposure in rabbits (0–15 mg/kg, day 7–19) also gave certain fetal effects (delayed ossification, weight decrease, retrocaval ureter, fussed sternebrae) at doses (2 and 5 mg/kg) which did not affect the dam. Finally, pentaBDE exposure decreased both maternal and fetal weights at relatively high doses (100–200 mg/kg)

Interactions with hormones and hormone receptors

Steroid hormones

No data have been found on interaction of PBDEs with steroid hormones.

Thyroid hormones

It is a common finding in subacute, subcronic and cronic toxicity studies on PBDEs that the thyroid gland is enlarged and exhibits follicular cell hyperplasia (see IPCS 1994a). In one study the thyroid hyperplasia was also correlated to adenoma and carcinoma formation (IPCS 1994a). Related to this, recent studies show that PBDEs lower the serum total and free T4 in mice and rats. Significantly decreased serum T4 concentrations were found in C57BL/6 mice treated orally with a commercial pentaBDE mixture (DE-71) both after acute exposure to 0.8–500 mg/kg and after daily exposure for 14 days to total doses of 250–1000 mg/kg (Fowles et al., 1994). Similarly, Sprague-Dawley rats and C57BL/6 mice given daily oral doses of Bromkal 70 (containing about 40% of tetraBDE) for 14 days at total dose levels of 0, 250 or 500 mg/kg (0, 18 or 36 mg/kg/day) showed significantly and dose-dependently decreased levels of plasma total T4 (Darnerud and Sinjari 1996). Rats proved to be more sensitive in this respect

than mice. The plasma thyroid stimulating hormone (TSH) concentrations, measured only in rats, were largely unaffected. The pure congener 2,2',4,4'-tetraBDE was equally or more potent in decreasing serum total T4 than the commercial pentaBDE in mice, and it could therefore be concluded that this effect is related rather to PBDEs than to their impurities. In addition, a technical grade PCB mixture Aroclor 1254 (containing about 5% pentaCB) and the pure congener 2,3,3',4,4'-pentaCB (a mono-ortho PCB with certain Ah binding affinity) were somewhat more potent than the PBDEs in causing these effects in mice. These studies demonstrated that the thyroid homeostasis is a sensitive target of PBDEs. Consequently, altered thyroid function could be an important mechanism in PBDE toxicity.

In man, indication of thyroid hormone disturbances has also been reported. Workers exposed to polybrominated biphenyls (PBBs) and PBDEs including decaBDE during manufacture were reported to have a higher than normal prevalence of primary hypothyroidism and a significant reduction of conducting velocities in sensory and motor neurons, but no other neurological or dermatological changes (Bahn et al., 1980; cf. IPCS 1994b). It was not possible to conclude whether these changes were attributed to PBB or PBDE exposure, but at least no decaBDE could be detected in serum of the exposed workers.

Retinoids

Reduced hepatic and pulmonary retinoid levels were found in male rats exposed daily to Bromkal-70 (2.5, 25 or 250 mg/kg body weight, p.o.) for 28 days, whereas renal levels were increased (Håkansson, unpublished results). The observations were dose-related. The responses in renal and pulmonary retinoid levels were less pronounced in female rats.

CPs

The toxicity of chlorinated paraffins (CPs) of various chain lengths in mammals has been well studied and is low following both acute and repeated exposures. In repeated high dose studies, the thyroid is among the primary target organs for the toxicity of the CPs. CPs of short chain length are acutely toxic to freshwater and salt water invertebrates and show long-term toxicity to algae, aquatic invertebrates and fish. Based on limited available data, the acute toxicity of CPs in birds seems to be low.

The existing, rather limited, amount of data from experimental studies on effects of CPs on reproduction, development and endocrine systems are given in more detail below. The following criteria documents and risk assessments concern all aspects of CP toxicity (IPCS 1989, 1996).

Reproductive and developmental effects

In the only identified reproduction study, no adverse reproductive effects were reported following exposure of rats to an intermediate chain length CP with 52 % chlorine (Serrone et al.. 1987). However, survival and body weight of the exposed pups were reduced. In a limited number of studies of the developmental effects of the short, medium and long chain CPs, adverse effects in the offspring were observed for the short chain compounds only, at maternally toxic doses in rats (IRCD 1982). For the medium and long chain compounds, no effects on the offspring were observed even at very high doses (IRDC1983a,b,c, 1984a,b).

Reduced ovary weights were observed in rats exposed to 3 g/kg bw/day of a short chain length CP (58% chlorine) (IRDC 1981a,b).

Interactions with hormones and hormone receptors

Steroid hormones

No data have been found on interaction of CPs with steroid hormone pathways.

Thyroid hormones

Two short chain CPs (56 and 58% chlorine) at 10 g/kg bw/day reduced both free and total plasma thyroxine (T4) levels in male rats, while there was no effect on T3 plasma levels (Wyatt et al., 1993). Increased thyroid weight and mild histopathological changes in thyroid glands were noted in rats exposed to medium-chain CPs (52% chlorine) in the diet for 13 weeks at doses as low as 4.2 mg/kg bw/day in female rats (Poon et al., 1995).

Retinoids

Reduced hepatic retinoid levels were found in rats exposed to medium-chain CPs (52% chlorine) in the diet for 13 weeks (Poon et al., 1995). The effect was dose-related and the relative potency to reduce hepatic vitamin A was 0.0000005, as compared to TCDD.

HALOGENATED PHENOLS AND BENZENES

Exposure to hexachlorobenzene (HCB) has been shown to result in a broad spectrum of effects, including enlarged liver, porphyria (disturbed haem synthesis) and immunosuppression. For most effects of HCB it appears that metabolism is a prerequisite.

HCB and pentachlorophenol (PCP) have adverse effects on reproduction and they affect steroid hormone levels. PCP binds with high affinity to thyroid hormone transporting serum proteins.

Reproductive and developmental effects

In rats, HCB reduced the litter size, increased the number of stillborn, and reduced the number of pups that survived to weaning (Grant et al., 1977). PCP, which is a major metabolite of HCB, has also been shown to reduce the litter size and increase the number of stillborn in rats (Exon and Koller 1982).

Interactions with hormones and hormone receptors

Steroid hormones

HCB has been observed to induce testosterone 16α- and 16β-hydroxylases in rats (Haake et al., 1987). Reduced estrogen levels have been observed in rhesus monkeys treated with HCB (Muller et al., 1978). HCB treatment of cynomolgus monkeys (*Macaca fascicularis*) resulted in suppression of serum progesterone during the luteal phase but not during the follicular or periovulatory phases (Foster et al., 1992). There was also a dose-dependent increase in menstrual cycle length and a reduction in estrogen levels in response to HCB (Foster et al., 1995)

Thyroid hormones

Treatment of rats with either HCB or pentachlorobenzene (0.03% in the feed for 13 weeks) resulted in disturbances of the thyroid system (den Besten et al., 1993). Reduced plasma T3 and T4 levels were observed in calves exposed to PCP (Hughes et al., 1985). In rats, both HCB and PCP exposure resulted in reduced uptake of T4 into cerebrospinal fluid and brain tissue (van Raaij et al., 1991). PCP has been shown to bind to human TTR with about twice the affinity of T4 (van den Berg 1990). In rats, PCP was a much more potent competitor for the T4-binding sites of serum carriers than HCB (van Raaij et al., 1991).

Retinoids

In rats treated with HCB or pentachlorobenzene (0.03% in the feed for 13 weeks) decreased hepatic levels of retinoids were observed (den Besten et al., 1993).

PCNs

Polychlorinated naphthalenes (PCNs) are of moderate acute toxicity to fish and of moderate to high toxicity in other aquatic organisms. From the available very limited amount of data on toxicity in terrestrial animals it seems as if PCNs have a chronic toxicity potential in mammals.

Data from experimental studies on effects of PCN on reproduction, development and endocrine systems are given below. The following documents cover various aspects of PCN toxicity and risk assessment (US/EPA 1975, 1980, Crookes and Howe 1993).

Reproductive and developmental effects

Halowax 1014 given to male and female chickens had no effect on the rate of egg production, but there was a marked and dose-related decrease in hatchability of the eggs (Pudelkiewicz et al., 1959). When Halowax 1014 was injected into chicken eggs at a dose of 3.0 mg/kg egg, 33% of the embryos died. The same dose of a mixture of 1,2,3,5,6,7-hexachloronaphthalene and 1,2,3,4,6,7-hexachloronaphthalene caused a mortality rate of 50% (Engwall et al., 1994).

Interactions with hormones and hormone receptors

Steroid and thyroid hormones

No data have been found on interaction of PCNs with steroid or thyroid hormone systems.

Retinoids

Much of the concern over PCN toxicity has focused on bovine hyperkeratosis or the X-disease. A cause of this disease appears to be the interference of the higher chlorinated PCN congeners with biotransformation of carotene to vitamin A i.e. retinoids (reviewed by Olson 1969). Studies both in cattle (Olafson 1947) and pigs (Link et al., 1958) have demonstrated decreased serum vitamin A levels as the disease progresses.

ALKYL PHENOLS

Alkyl phenols (APs) are toxic to aquatic organisms and poorly degraded in water. Nonylphenol (NP) is the most studied of the APs and is together with octylphenol (OP) determined to be among the most potent estrogenic compounds within this group of chemicals. OP and NP produced the same effect as estrogen *in vitro* systems, but at several orders of magnitude higher concentrations. Likewise, NP and OP have proven to be estrogenic *in vivo*, inducing vitellogenin synthesis in fish. Furthermore, OP increased the uterine weight and reduced the testis size and sperm production in rats exposed during gestation and lactation.

Far-reaching plans have been instituted to limit and ultimately phase out the use of NP in Sweden.

Data from experimental studies on effects of APs on reproduction, development and endocrine systems are given below. In addition, the following documents and risk assessments concern many aspects of AP toxicity: Nilsson 1996, TemaNord 1996, KemI 1997.

Reproductive and developmental disturbances

Subcutaneous doses of 50 mg NP (approximately 370 mg/kg bw) administered to rats on day 19 and 20 after ovariectomy induced a significantly higher endometrial mitotic index compared to controls (Soto et al., 1991). The response was one third of that induced by 1.25 μg estradiol-17β (approx. 10 μg/kg bw). Decreased sperm count at adulthood (90–95 days of age) and reduced relative testis weight were seen in offspring to rats exposed to OP-5-ethoxylate through drinking water during pregnancy (Sharpe et al., 1995). The decreases in testis weight and sperm count were observed at 100 and 1000 ppm, respectively.

Exposure of male rainbow trout to NP resulted in reduced gonadal size and sperm production (Jobling et al., 1996).

Interactions with hormones and hormone receptors

Steroid hormones

The alkylphenolic compounds were shown already in 1938 (Dodds and Lawson, 1938) shown to possess estrogenic activity. Although less potent than 17β-estradiol, both OP and NP are capable of displacing 17β-estradiol from ER in rainbow trout and activate transcription in estrogen receptor transfected MCF-7 cells (White et al., 1994). NP also induced vitellogenesis in male fish (MacLatchy et al., 1995).

NP and OP caused proliferation of estrogen-sensitive MCF-7 human breast cancer cells (Soto et al., 1995). Their potencies were 0.003% and 0.03% of that of estradiol, respectively. Other short chained alkyl phenols investigated in this test were less active.

NP, NP-carboxylic acid, and NP-diethoxylate were all capable of stimulating vitellogenin expression in trout hepatocytes, growth in two estrogen-responsive human breast cancer cell lines (MCF-7 and ZR-75), and transcription of the estrogen receptor in transfected cells (White et al., 1994). The action of these compounds appeared to be mediated by the estrogen receptor, since their effects depended on its presence and were blocked by estrogen antagonists.

APs have estrogenic effects and have also been shown to bind directly to the estrogen

receptor (White et al., 1994). NP is the most used/studied of the APs and is together with OP determined to be among the most potent estrogenic compounds within this group of chemicals (White et al., 1994). Soto et al., (1991) showed that the *para*-substituted *tert*-butylphenol had estrogenic properties in MCF-7 cells, whereas the *ortho*- and *meta*-substituted tert-butylphenols were inactive.

The effects of NP in rainbow trout hepatocyte cultures have been shown to be transient (Flouriot et al., 1995). Thus, maximum levels of ER mRNA were observed 5 hours after treatment of hepatocytes with 10 μM NP and the levels decreased thereafter. A slight induction of vitellogenin mRNA was noted when the ER mRNA level was maximal. This transient induction of both ER and vitellogenin was suggested to be due to rapid metabolism of NP by the hepatocytes.

Besides vitellogenin, the egg shell proteins have been suggested to be good indicators of NP exposure (Arukwe et al., 1997). Following injection of 4-NP in Atlantic salmon, vitellogenin was induced at 125 mg/kg while the egg shell proteins were induced already at 1 mg 4-NP/kg. This indicates that induction of egg shell proteins may be a more sensitive response to estrogenic compounds than vitellogenin induction.

Thyroid hormones and retinoids

No data have been found on interaction of APs with either the thyroid hormone or retinoid pathways.

PHTALATES

Phtalates comprise a large group of structurally related compounds used primarily as plasticizers. Di(2-ethylhexyl) phthalate (DEHP) accounts for over half of the total use ofphtalates, and it is also the most well-studied of these compounds. Most studies indicate that although these substances are able to induce adverse effects on reproduction parameters, their potency is low. An exception to that might be exposure in utero and/or via maternal milk. Furthermore, there are studies demonstrating that phtalates interfere with steroid and thyroid hormone systems.

Data from experimental studies on effects ofphtalates on reproduction, development and endocrine systems are given below. Recent criteria documents and risk assessments concerning all aspects of phthalate toxicity include (IPCS 1992, ATSDR 1993a,b, 1994, KemI 1994, TemaNord 1996). In addition, a new document on DEHP is being prepared by the Swedish National Chemicals Inspectorate.

Reproductive and developmental disturbances

A large number of studies with DEHP (at doses higher than 100 mg/kg bw/day) have shown decreased weights of testis, seminal vesicles, epididymis, and prostate gland as well as testicular atrophy in experimental animals (reviewed in ATSDR 1993a, and IPCS 1992). Decreased male fertility, damaged spermatogenic cells and abnormal sperm have also been observed in DEHP-treated rats and mice. In most of these studies, adult males were exposed. In female laboratory animals, exposure to DEHP (at

doses higher than 100 mg/kg bw/day) caused decreased ovarian and uterine weights as well as infertility.

Pregnant rats and their offspring were given butyl benzyl phthalate (BBP; 1 mg/L) in the drinking water from two weeks before mating until the pups were 22 days old (Sharpe et al., 1995). When the male rat pups reached maturity, it was found that BBP-treated rats had significantly lowered testicular weight (9% reduction) and sperm production (17% reduction) than control rats. In another study, male and female rats were exposed to BBP before mating and throughout pregnancy (Piersma et al., 1995). At 1000, but not at 500, mg/kg bw/day, adverse effects were found on spermatogenesis and on pregnancy and litter parameters. Adverse testis effects were seen in male rats exposed to approximately 1 g BBP/kg bw/day (TemaNord 1996)

When male and female mice were exposed to several grams of diethyl phthalate/kg bw/day (ATSDR 1993b), sperm concentration and the number of live pups per litter were significantly decreased. No adverse effects on fertility were observed.

In mice, high doses of di-n-octyl, di-n-propyl or di-n-pentyl pthalate (several grams/kg bw/day over two generations) caused reductions in percent fertile pairs or on number of litters/pair, inhibited fertility, and/or reduced seminal vesicle weights (Heindel et al., 1989; Morrissey et al., 1989; NTP 1985; ATSDR 1994). No other effects on testis or sperm parameters were seen. Females or pups were not affected.

In short-term rat studies, large doses (several grams/kg bw and day) of di-n-butyl, di-n-pentyl, di-n-hexyl phtalate seemed to induce adverse testicular effects, while methyl, ethyl, n-propyl, di-n-octyl and n-heptyl phtalate had no such effects (Foster et al., 1980, Gray and Butterworth 1980, Mann et al., 1985, Oishi and Hiraga 1980; ATSDR 1994. Fukuoka et al., 1993; TemaNord 1996).

Birds fed diets with 10 ppm of dibutyl phtalate produced eggs with reduced shell thickness and size (Peakall 1974; TemaNord 1996). DEHP had no such effects.

Interactions with hormones and hormone receptors

Steroid hormones

DEHP has negative effects on Sertoli cells both *in vivo* and *in vitro* (IPCS 1992). The results of in vitro studies suggest that DEHP, dibutyl and dipentyl phthalate, but not dimethyl phthalate, can render Sertoli cells less sensitive to endogenous hormones; this effect may be involved in the mechanism underlying the effects on testicles (Grasso et al., 1993).

Butyl benzyl phthalate caused proliferation of estrogen-sensitive MCF-7 human breast cancer cells (Soto et al. 1995). Its potency was 0.0003% of that of estradiol.

Butyl benzyl phthalate and dibutyl phthalate have been shown to have estrogenic effects in human breast tumor cells; stimulating growth and transcriptional activity of the estrogen receptor (reviewed in Danish Environmental Protection Agency 1995). The potencies compared to estradiol are 10–5 and 10–6, respectively.

Thyroid hormones

In rats, chronic exposure to DEHP (1000 mg/kg bw/day) induced a persistent decrease in serum thyroxine (T4) levels (reviewed in ATSDR 1993a). The serum levels of triiodothyronine (T3) did not change. Since the decreased T4 levels apparently were not consistent with hyperactivity of the secretory cells, these changes were interpreted to be due to increased clearance or degradation of the thyroid hormone.

Retinoids

No data have been found on interaction ofphtalates on retinoid pathways.

BISPHENOL-A AND TETRABROMOBISPHENOL-A

Studies *in vitro* have shown that bisphenol-A is an estrogen receptor agonist. Bisphenol-A is used in epoxy-lacquered coating of cans and may be released from these. Brotons and coworkers (1995) observed that some foods (peas, artichokes, green beans, mixed vegetables, corn, mushrooms) from such cans exhibited significant estrogenic activity in the MCF-7 cell line E-Screen test. Bisphenol-A has also been found to be released from polycarbonate plastic during autoclaving (Krishnan et al., 1993).

Reproductive and developmental effects

Exposure of rats (160–640 mg/kg/day) and mice (500–1250 mg/kg/day) to bisphenol-A resulted in fetal toxicity in mice but not in rats (Morrissey et al., 1987).

A significant increase in uterine water content was observed in rats 18 h after an injection of 50 or 100 mg bisphenol A per kg bw (TemaNord 1996).

Interactions with hormones and hormone receptors

Steroid hormones

Bisphenol A stimulates the growth of human breast cancer cells (Brotons et al., 1995, Krishnan et al., 1993; TemaNord 1996). It has also been shown that bisphenol-A competes with estrogen binding to ER with an affinity of 1:2000 compared to estradiol-17β (Krishnan et al., 1993). Bisphenol-A, at a concentration of 10–25 mM, also induced the progesterone receptor in MCF-7 cells. This induction of the progesterone receptor could be blocked by tamoxifen.

Thyroid hormones

TBBP-A has been shown to compete in vitro with thyroxine for the binding site on TTR (Brouwer, personal communication). No data have been found about effects of bisphenol A on thyroid hormone pathways.

Retinoids

No data have been found on interaction of bisphenol A with retinoid pathways.

CHEMICAL INTERACTIONS

It has been suggested that environmental chemicals having estrogenic effects may act in concert. Thus, when OH-PCBs were applied together at subthreshold levels for each individual compound, they induced sex reversal in turtle eggs incubated at male-producing temperatures (Bergeron et al., 94).

In a study on rainbow trout hepatocytes, additive effects on vitellogenin production were found for a combination of NP, NP ethoxylate and NP carboxylic acids (Harries et al.,1995).

Mixtures of DDT metabolites reduced estradiol binding to estrogen receptor prepared from American alligator oviduct in an additive fashion, whereas combinations

of chemicals identified in Lake Apopka appeared to have a greater than additive effect (Vonier et al.,1996).

The combined effect of two OH-PCBs (2',4',6',-trichloro-4-biphenylol and 2',3',4',5'-tetrachloro-4-biphenylol) on estrogen mediated gene activation indicates that they act synergistically (Arnold et al., 996). These authors also found that dieldrin, toxaphene, endosulfan and chlordane acted synergistically when tested in different combinations in transgenic yeast cells. The results from these studies have, however, been questioned (Kaiser 1997).

Recently, the estrogenic effects of dieldrin and endosulfan were tested in a yeast cell estrogen receptor assay and in a uterus growth assay (Ashby et al., 997). Using methoxychlor (a known estrogen agonist) at a concentration that gave a submaximal estrogenic response, this study did not indicate that either dieldrin or endosulfan activated the system when added as a mixture. Neither did they alone or in combination with methoxychlor enhance the signal achieved with metoxychlor alone. Thus, these results do not support the hypothesis that synergism between chemicals will lead to estrogenic activity.

Subsequent experiments performed to confirm the study by Arnold et al., (1996) has led to the withdrawal of the original paper (McLachlan, 1997). It can therefore not be concluded at this time that environmental chemicals act in synergy to activate the estrogen receptor.

SUMMARY

Experimental data clearly demonstrates that most of the chemical compounds addressed in this document can impair vertebrate reproduction, while effects on developmental processes have been demonstrated by a more limited number of compound classes.

Effects on reproduction (e.g. decreased fertility and survival of the offspring) and reproductive tissues (e.g. decreased testes weight, delayed onset of male puberty, delayed vaginal opening, prolonged estrus cycle) in experimental studies with mammalian species have been observed following exposure to DDT, lindane, PCDDs/PCDFs, PCBs, HCB, PCP, andphtalates. Corresponding effects in birds include egg shell thinning and decreased hatchability following exposure to DDT, lindane, PCBs, HCB, and PCN. In fish, impaired reproduction (decreased hatchability, delayed spawning) has been observed following exposure to DDT and PCB, whereas decreases in testis weight and sperm production have been observed in fish exposed to nonylphenol. Decreased weights of testis, ovaries, and uterus have been observed in rats exposed tophtalates.

Exposure to DDT, PCDDs/PCDFs, or PCBs during development, i.e. *in utero*, has

resulted in behavioral and learning deficits later in life. Altered mating behavior and feminization of the male offspring has been observed among birds exposed to DDT, and mammals exposed to PCDDs/PCDFs and PCBs. Impaired learning and auditory deficits are additional findings in the PCB-exposed offspring. Male rats exposed to octylphenol or butylbenzylphthalate *in utero* exhibited decreases in testis weight and sperm production at maturity.

The observations in experimental animals are consistent with some of the documented and discussed effects in humans and wildlife (see Chapter 4). However, cancers of the testes, prostate, or breast, which are increasing in the human population, have not been observed in experimental cancer studies on any of the compounds addressed in this evaluation. In contrast tumors of the thyroid gland have been observed in rodents exposed to TCDD or CPs. Decreased incidences of tumors in the pituitary, adrenal and mammary glands, and uterus have also been observed in TCDD-exposed rats.

The mechanisms behind the observed effects in experimental animals are largely unknown, and it is not clear whether or not the primary effect, for any of these contaminants, is due to endocrine disruption. However, there is extensive experimental evidence demonstrating that, among these groups of compounds, there are a large number of potent modulators of the steroid, thyroid, or retinoid systems (Table 6.1).

Direct interaction with steroid hormone receptors has been demonstrated in *in vitro* systems for many of the compounds (e.g. OH-PCBs, DDT, alkyl phenols, andphtalates). These compounds also induce estrogenic responses in *in vitro* systems (e.g. proliferation of MCF-7 cells) but only in a few cases has an estrogenic response by these compounds been validated in *in vivo* models (e.g. vitellogenin synthesis). PCDDs/Fs and dioxin like PCBs are potent anti-estrogens; they do not bind to the estrogen receptor and they inhibit estrogen induced responses *in vitro* (MCF-7 cells) and *in vivo* (vitellogenin synthesis).

Interactions between environmental pollutants and thyroid hormones include direct binding of the pollutant or its metabolite to transthyretin, which is the plasma transport protein for thyroxin (and retinol), and altered tissue levels of T3 and T4. Compounds that bind to transthyretin *in vitro* include (DDT, PCDDs/Fs, certain PCBs (OH-PCBs), and bisphenol A.

All persistent organic halogens surveyed in this evaluation alter tissue levels of retinoids or critical steps in the retinoid metabolism. Effects on the retinoid system has been demonstrated in a large number of species and the response is observed following *in utero* exposure, although most studies were made in adult animals. The most potent retinoid disrupting compounds are the PCDDs/Fs and coplanar PCB congeners. The potency of individual PCDD, PCDF, and PCB congeners, to decrease hepatic vitamin A levels, is related to the chemical structure of the compound. This

Table 6.1. Interactions of some environmental pollutants with hormonal systems.

Chemical group	Interaction with hormone system:		
	steroid	thyroid	retinoid
Pesticides			
– DDT	+	+	+
– lindane	+	+	+
PCDD/F	+	+	+
PCB	+	+	+
PBDE	nd	+	+
Chlorinated paraffins	nd	+	+
Halogenated benzenes and phenols	+	+	+
Polychlorinated naphthalenes	nd	nd	+
Alkyl phenols	+	nd	nd
Phthalates	+	-	nd
Bisphenol A	+	nd	nd

+ interaction (agonistic and/or antagonistic);
– no effect
nd no data found

effect may be associated to the affinity of these compounds for the Ah-receptor. In contrast to TCDD, which increases plasma retinoid levels, there are some PCB congeners, which decrease serum vitamin A levels. This effect may be associated to the ability of hydroxylated PCB-metabolites to bind to transthyretin and thereby interfere with the serum transport of vitamin A. In addition, PBDEs, CPs, DDT, lindane, and PCN (with no or low affinity for the Ah-receptor), also affect tissue retinoid levels,possibly through other or mixed mechanisms.

In addition to the hormones evaluated, many other regulatory systems, such as the immune and nervous systems, are involved during developmental processes and reproduction. Together they form a central unifying regulatory system used by cells and tissues to integrate information relating to their state of differentiation and proliferation. Numerous events in these regulatory processes may be impaired, yet there are very few established test-systems for these processes. Furthermore, the endpoints in use are generally of an integrated character and does not give any information as to the possible target event or to the mechanism of action. It is obvious that

there are large data gaps both with regard to the general developmental and reproductive physiology and even more so with regard to the toxicology of these processes.

Based on this knowledge it is not surprising that EDSs can belong to completely different classes of chemicals, having markedly different chemical and physical properties. It is also likely that in the adult, individual mechanisms for controlling modest fluctuations of hormones are in place and protect against manifest adverse consequences of endocrine disrupting chemicals. In contrast, even minor disturbances in the endocrine systems during embryogenesis and fetal development are likely to have serious consequences for the proper development of both the neuronal and reproductive systems.

References

Abbott, B.D. and Birnbaum, L.S. (1989) Cellular alterations and enhanced induction of cleft palate after coadministration of retinoic acid and TCDD. Toxicol. Appl. Pharmacol., 99:287-301.

Abbott, B.D., Birnbaum, L.S. and Pratt, R.M. (1987) TCDD-induced hyperplasia of the ureteral epithelium produces hydronephrosis in murine fetuses. Teratology, 35:329-334.

Adams, N.R. (1983) Sexual behaviour of ewes with clover disease treated repeatedly with oestradiol benzoate or testosterone propionate after ovariectomy. J. Reprod. Fert. 68:113-117.

Adlercreutz, H., van der Wildt, J., Kinzel, J., Attalla, H., Wahala, K., Makela, T., Hase, T. and Fotsis, T. (1995) Lignan and isoflavonoid conjugates in human urine. J. Steroid. Biochem. Mol. Biol. 52:97-103.

Ahlborg, U.G., Holmstedt, B. and Håkansson, H. (1989) Polychlorinated dibenzo-para-dioxins and dibenzofurans. Environmental Health Criteria 88. World Health Organization, Geneva.

Ahlborg, U.G., Waern, F. and Håkansson, H. (1987) Interactive effects of PCDDs and PCDFs occurring in human mother's milk. Chemosphere 16:1701-1706.

Allen, J.R. (1975) Response of the nonhuman primate to polychlorinated biphenyl exposure. Fed Proc. 34:1675-1679.

Allen, J.R., Lambrecht, L.K. and Barsotti, D.A. (1978) Effects of polybrominated biphenyls in nonhuman primates. J. Am. Vet. Med. Assoc., 173:1485-1489.

Allred, P.M. and Strange, J.R. (1977) The effects of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on developing chicken embryos. Arch. Environm. Contam. Toxicol., pp 483-489.

Arnold, S.F., Klotz, D.M., Collins, B.M., Vonier, P.M., Guillette, L.J., Jr. and McLachlan, J.A. (1996) Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science. 272:1489-1492.

Arukwe, A., Knudsen, F.R. and Goksoyr, A. (1997) Fish zona radiata (eggshell) protein: A sensitive biomarker for environmental estrogens. Environ. Health. Perspect. 105:418-422.

Ashby, J., Lefevre, P. A., Odum, J., Harris, C. A., Routledge, E. J. and Sumpter, J. P. (1997) Synergy between synthetic oestrogens? Nature. 385:494.

ATSDR. (1993a) Toxicological profile for di(2-ethylhexyl) phthalate. U.S. Depart-

ment of Health & Human Services, TP-92/05.

ATSDR. (1993b) Toxicological profile for diethyl phthalate. Draft for public comment. U.S. Department of Health & Human Services.

ATSDR. (1994) Toxicological profile for di-n-octyl phthalate. Draft for public comment. U.S. Department of Health & Human Services.

Azais, V., Arand, M., Rauch, P., Schramm, H., Bellenand, P., Narbonne, J.F., Oesch, F., Pascal, G. and Robertson, L.W. (1987) A time-course investigation of vitamin A levels and drug metabolizing enzyme activities in rats following a single treatment with prototypic polychlorinated biphenyls and DDT. Toxicology 44:341-354.

Bahn, A.K., Mills, J.L., Synder, P.J., Gann, P.H., Houten, L., Bialik, O., Hollmann, L. and Utiger, R.D. (1980) Hypothyroidism in workers exposed to polybrominated biphenyls. N. Engl. J. Med. 302:31-33.

Bank, P.A., Cullum, M.E., Jensen, R.K. and Zile, M.H. (1989) Effect of hexachlorobiphenyl on vitamin A homeostasis in the rat. Biochim Biophys Acta 990:306-314.

Barsotti, D.A. and Van Miller, J.P. (1984) Accumulation of a commercial polychlorinated biphenyl mixture (Aroclor 1016) in adult rhesus monkeys and their nursing infants. Toxicology. 30:31-44.

van den Berg, K.J. (1990) Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin. Chem Biol Interact. 76:63-75.

Berkers, J.A.M., Hassing, G.A.M., Brouwer, A. and Blaauboer, B.J. (1994) Effects of retinoic acid on the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced differentiation of *in vitro* cultured human keratinocytes. Toxicology in Vitro 8: 605-608.

van Birgelen, A.P.J.M., van der Kolk, J., Fase, K.M., Bol, I., Poiger, H., van den Berg, M. and Brouwer, A. (1994) Toxic potency of 2,3,3',4,4',5-hexachlorobiphenyl relative to and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a subchronic feeding study in the rat. Toxicol. Appl. Pharmacol. 126: 202-213.

Bergeron, J.M., Crews, D. and McLachlan, J.A. (1994) PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ. Health Perspect., 102:780-781.

den Besten, C., Bennik, M. H., Bruggeman, I., Schielen, P., Kuper, F., Brouwer, A., Koeman, J. H., Vos, J. G. and Van Bladeren, P. J. (1993). The role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis: a comparison with pentachlorobenzene in a 13-week feeding study. Toxicol. Appl. Pharmacol. 119: 181-194.

Birnbaum, L.S., Harris, M.W. Stocking, L.M. Clark, A.M. and Morrissey, R.E. (1989) Retinoic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) selectively enchance teratogenesis in C57BL/6N mice. Toxicol. Appl. Pharmacol., 98:487-500.

Bitman, J., Cecil, H.C. and Harris, S.J. (1972) Biological effects of polychlorinated biphenyls in rats and quail. Environ Health Perspect 1:145-149.

Bock, K.W. (1991). Roles of UDP-glucuronosyltransferases in chemical carcinogenesis. Crit. Rev. Biochem. Mol. Biol. 26:129-150.

Brotons, J.A., Olea Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. (1995) Xenoestrogens released from lacquer coatings in food cans. Environ-Health-Perspect. 103:608-612.

Brouwer, A. (1987) Interference of 3,4,3',4'-tetrachlorobiphenyl in vitamin A (retinoids) metabolism: Possible implications for toxicity and carcinogenicity of polyhalogenated aromatic hydrocarbons. University of Leiden, Leiden.

Brouwer, A, and van den Berg, K.J. (1986) Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex, carrying both retinol and thyroxin. Toxicol Appl Pharmacol. 85: 301-312.

Brouwer, A., van den Berg, K.J. and Kukler, A. (1985) Time and dose responses of the reduction in retinoid concentrations in C57BL/Rij and DBA/2 mice induced by 3,4,3',4'-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol. 78:180-189.

Brouwer, A., Håkansson, H., Kukler, A., van den Berg, K.J. and Ahlborg, U.G. (1989) Marked alterations in retinoid homeostasis of Sprague- Dawley rats induced by a single i.p. dose of 10 µg/kg of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology. 58:267-283.

Brunstrom, B. (1988) Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3',4,4'-tetrachlorobiphenyl. Poult. Sci. 67: 52-57.

Brunstrom, B., Hakansson, H. and Lundberg, K. (1991) Effects of a technical PCB preparation and fractions thereof on ethoxyresorufin O-deethylase activity, vitamin A levels and thymic development in the mink (*Mustela vison*). Pharmacol. Toxicol. 69:421-426.

Bulger, W.H. and Kupfer, D. (1977) Inhibition of the 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane (o,p'DDT)- and estradiol-mediated induction of rat uterine ornithine decarboxylase by prior treatment with o,p'DDT estradiol, and tamoxifen. Arch. Biochem. Biophys. 182:138-146.

Burlington, H. and Lindeman, V.F. 1950 Effects of DDT on testes and secondary sex characters of White Leghorn cockerels. Proc Soc Exp Biol Med 74:48-51.

Bryan, T.E., Gildersleeve, R.P. and Wiard, R.P. (1989) Exposure of Japanese quail embryos to o,p'-DDT has long-term effects on reproductive behaviors, hematology, and feather morphology. Teratology. 39:525-535.

Cassidy, A. and Bingham, S. (1995) Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. British J. Nutr. 74:587-601.

Cassidy, A., Bingham,S. and Setchell, K.D.R. (1994) Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal woman. Am. J. Clin. Nutr., 60:333-340.

Cecil, H.C., Harris, S.J., Bitman, J. and Fries, G.F. (1973) Polychlorinated biphenyl-induced decrease in liver vitamin A in Japanese Quail and rats. Bull Environ. Contam. Toxicol. 9:179-185.

Chahoud, I., Hartmann, J., Rune, G.M. and Neubert, D. (1992) Reproductive toxicity and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects of single doses on the testis of male rats. Arch. Toxicol. 66:567-72.

Charkravarty, S. and Lahiri, P. (1986) Effect of lindane on eggshell characteristics and calcium level in the domestic duck. Toxicology. 42:245-258.

Chen, L.C., Berberian, I., Koch, B., Mercier, M., Azais-Braesco, V., Glauert, H.P., Chow, C.K. and Robertson, L.W. (1992) Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: Structureactivity relationships. Toxicol. Appl. Pharmacol. 114:47-55.

Chowdhury, A.R. and Gautam, A.K. (1994) Steroidogenic impairment after lindane treatment in male rats. Sangyo Ika Daigaku Zasshi. 16:145-52.

Chu, I., Villeneuve, D.C., Yagminas, A., LeCavalier, P., Håkansson, H., Ahlborg, U.G., Valli, V.E., Kennedy, S.W., Bergman, Å., Seegal, R.F. and Feeley, M. (1995) Toxicity of PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 118 (2,3',4,4',5-pentachlorobiphenyl) in the

rat following subchronic dietary exposure. Fund Appl Toxicol. 26:282-292.

Chu, I., Villeneuve, D.C., Yagminas, A., LeCavalier, P., Poon, R., Feeley, M., Kennedy, S.W., Seegal, R.F., Håkansson, H., Ahlborg, U.G. and Valli, V.E. (1994) Subchronic Toxicity of 3,3',4,4',5-Pentachlorobiphenyl in the Rat .1. Clinical, Biochemical, Hematological, and Histopathological Changes. Fund Appl Toxicol. 22:457-468.

Chu, I., Villeneuve, D.C., Yagminas, A., LeCavalier, P., Poon, R., Feeley, M., Kennedy, S.W., Seegal, R.F., Håkansson, H., Ahlborg, U.G., Valli, V.E. and Bergman, Å. (1996b) Toxicity of 2,2',4,4',5,5'-hexachlorbiphenyl in rats: Effects following 90-day oral exposure. J Appl Toxicol. 16:121-128.

Chu, I., Villeneuve, D.C., Yagminas, A., LeCavalier, P., Poon, R., Hakansson, H., Ahlborg, U.G., Valli, V.E., Kennedy, S.W., Bergman, A., Seegal, R.F. and Feeley, M. (1996c) Toxicity of 2,4,4'-trichlorobiphenyl in rats following 90-day dietary exposure. J. Toxicol. Environ. Health. 49:301-318.

Cooke, P.S., Zhao, Y.D. and Hansen, L.G. (1996) Neonatal polychlorinated biphenyl treatment increases adult testis size and sperm production in the rat. Toxicol. Appl. Pharmacol. 136:112-117.

Cooper, R.L., Chadwick, R.W., Rehnberg, G.L., Goldman, J.M., Booth, K.C., Hein, J.F. and McElroy, W.K. (1989) Effect of lindane on hormonal control of reproductive function in the female rat. Toxicol. Appl. Pharmacol. 99:384-394.

Crookes, M.J. and Howe, P.D. (1993) Environmental hazard assessment: Halogenated naphthalenes. Toxic Substance Division Departement of the Environment.

Danish Environmental Protection Agency. (1995). Male reproductive health and environmental chemicals with estrogenic effects. Miljoprojekt nr. 290.

Darnerud, P.O. and Sinjari, T. (1996) Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroxine and TSH blood levels in rats and mice (abstract to Dioxin '96) Organohalogen Compounds 29: 316-319.

Denison, M.S., Fisher, J.M. and Whitlock, J.P., Jr. (1988) Inducible, receptor-dependent protein-DNA interactions at a dioxin-responsive transcriptional enhancer. Proc Natl Acad Sci USA. 85:2528-2532.

Dodds, E.C. and Lawson, W. (1938) Molecular structure in relation to oestrogenic activity. Compounds without a phenantrene nucleus. Proc. Royal Soc. London (Series B), 118:222-232.

Dohr, O., Vogel, C. and Abel, J. (1995) Different response of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB 231 cells. Arch. Biochem. Biophys. 321:405-412.

Engwall, M., Brunstrom, B. and Jakobsson, E. (1994) Ethoxyresorufin O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH)-inducing potency and lethality of chlorinated naphthalenes in chicken (*Gallus domestica*s) and eider duck (*Somateria mollissima*) embryos. Arch. Toxicol. 68:37-42.

Eriksson, P. and Fredriksson, A. (1996) Developmental neurotoxicity of four *ortho*-substituted polychlorinated biphenyls in the neonatal mouse. Environ. Toxicol. Pharmacol. 1:155-165.

Eriksson, P., Lundkvist, U. and Fredriksson, A. (1991) Neonatal exposure to 3,3',4,4'-tetrachlorobiphenyls: changes in spontaneous behaviour and cholinergic muscarinic receptors in adult mouse. Toxicol., 69:27.

Exon, J. H. and Koller, L. D. (1982) Effects of transplacental exposure to chlorinated phenols. Environ Health Perspect. 46:137-140.

Flouriot, G., Pakdel, F., Ducouret, B. and Valotaire, Y. (1995) Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellogenin gene expres-

sion. J. Mol. Endocrinol. 15:143-151.
Forsythe II, W.A. (1995) Soy protein, thyroid regulation and cholesterol metabolism. J Nutr 125:619S-623S.
Foster, P.M., Thomas, L.V., Cook, M.W. and Gangolli, S.D. (1980) Study of the testicular effects and changes in zinc excretion produced by some n-alkylphtalates in the rat. Toxicol Appl Pharmacol. 54:392-398.
Foster, W.G., McMahon, A., Younglai, E.V., Jarrell, J.F. and Lecavalier, P. (1995) Alterations in circulating ovarian steroids in hexachlorobenzene-exposed monkeys. Reprod. Toxicol. 9:541-548.
Foster, W.G., McMahon, A., Villeneuve, D.C. and Jarrell, J.F. (1992) Hexachlorobenzene (HCB) suppresses circulating progesterone concentrations during the luteal phase in the cynomolgus monkey. J. Appl. Toxicol. 12:13-17.
Fowles, J.R., Fairbrother, A., Baecher Steppan, L. and Kerkvliet, N.I. (1994) Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. Toxicology. 86:49-61.
Fry, D.M. and Toone, C.K. (1981) DDT-induced feminization of gull embryos. Science. 213:922-924.
Fukuoka, M., Kobayashi, T., Zhou, Y. and Hayakawa, T. (1993) Mechanism of testicular atrophy induced by di-n-butyl phthalate in rats. Part 4. Changes in the activity of succinate dehydrogenase and the levels of transferrin and ferritin in the Sertoli and germ cells. J. Appl. Toxicol. 13:241-246.
Gierthy, J.F., Spink, B.C., Figge, H.L., Pentecost, B.T. and Spink, D.C. (1996) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 12-O-tetradecanoylphorbol-13-acetate and 17 beta-estradiol on estrogen receptor regulation in MCF-7 human breast cancer cells. J. Cell. Biochem. 60:173-184.
Goldey, E.S., Kehn, L.S., Lau, C., Rehnberg, G.L. and Crofton, K.M. (1995). Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol. Appl. Pharmacol. 135:77-88.
Goodman, D.S. (1984) Plasma retinol binding protein. In: Sporn, M.B., Roberts, A.B., Goodman, D.S. (eds). Academic Press, Inc., Orlando, Florida, The Retinoids. 2:41-88.
Golub, M.S., Donald, J.M. and Reyes, J.A. (1991) Reproductive toxicity of commersial PCB mixtures: LOAELs from animal studies. Environ. Health Perspec., 94:245-253.
Gorski, J.R., Muzi, G., Weber, L.W., Pereira, D.W., Arceo, R.J., Iatropoulos, M.J. and Rozman, K. (1988) Some endocrine and morphological aspects of the acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol. Pathol. 16:313-320.
Gorski, J.R. and Rozman, K. (1987) Dose-response and time course of hypothyroxinemia and hypoinsulinemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. Toxicology. 44: 297-307.
Grant, D.L., Phillips, W.E. and Hatina, G.V. (1977) Effect of hexachlorobenzene on reproduction in the rat. Arch Environ Contam Toxicol. 5:207-216.
Grasso, P., Heindel, J. J., Powell, C.J. and Reichert, Jr. (1993) Effects of mono (2-ethylhexyl) phthalate, a testicular toxicant, on follicle-stimulating hormone binding to membranes from cultured Sertoli cells. Biology of Reproduction 48:454-459.
Gray, T.J. and Butterworth, K.R. (1980) Testicular atrophy produced by phthalate esters. Archives of Toxicology Supplement 4:452-455.
Gray, L.E., Kelce, M.R., Monosson, E. Ostby, J.S. and Birnbaum, L.S. (1995) Exposure to TCDD during development permanently alters reproductive function

in male LE rats and hamsters: reduced ejaculated and epididymal sperm numbers and sex accessory gland weights in offspring with normal androgenic status. Toxicol. Appl. Pharmacol., 131:108-118.

Gray, L.E., Ostby, J.S., Kelce, W. Marshall, R. Diliberto, J.J. and Birnbaum, L.S. (1993) Perinatal TCDD exposure alters sex differentiation in both female and male LE hooded rats. Dioxin '93, 13:337-340.

Greighus, Y.A. and Hannon, M.R. (1973) Distribution and biochemical effects of DDT, DDD and DDE in penned double-crested cormorants. Toxicol. Appl. Pharmacol. 26:483-494.

Haake, J., Kelley, M., Keys, B. and Safe, S. (1987) The effects of organochlorine pesticides as inducers of testosterone and benzo[a]pyrene hydroxylases. Gen. Pharmacol. 18:165-169.

Håkansson, H. and Ahlborg, U.G. (1985) The effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the uptake, distribution and excretion of a single oral dose of [11,12-3H]-retinylacetate and on the vitamin A status in the rat. J. Nutr. 115:759-711.

Håkansson, H., Ahlborg, U.G., Johansson, L. and Poiger, H. (1990) Vitamin A storage in rats subchronically exposed to PCDDs/PCDFs. Chemosphere 20:1147-1150.

Håkansson, H., Johansson, L., Manzoor, E. and Ahlborg, U.G. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the vitamin A status of Hartley guinea pigs, Sprague-Dawley rats, C57B1 6 mice, DBA 2 mice, and golden syrian hamsters. J. Nutr. Sci. Vitaminol. 37:117-138.

Harries, J.E., Jobling, S., Matthiessen, P., Sheahan, D.A. and Sumpter J.P. (1995) Effects of trace organics on fish, Phase 2. Department of the Environment Report. UK. FR/D 0022.

Heindel, J.J., Gulati, D.K., Mounce, R.C., Russell, S.R. and Lamb, J.C. (1989) Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. Fund Appl Toxicol. 12:508-518.

Henry, E.C. and Gasiewicz, T.A. (1987) Changes in thyroid hormones and thyroxine glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 89:165-174.

Higginbotham, G.R., Huang, A. Firestone, Verrett, J. Ress, J. and Campbell, A.D. (1968) Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-*p*-dioxin. Nature, 220:702-703.

Hook, M., Lindahl, U., Hallen, A. and Backstrom, G. (1975) Biosynthesis of heparin. Studies on the microsomal sulfation process. J. Biol. Chem. 250: 6065-6071.

Hughes, B.J., Forsell, J.H., Sleight, S.D., Kuo, C. and Shull, L.R. (1985) Assessment of pentachlorophenol toxicity in newborn calves: clinicopathology and tissue residues. J Anim Sci. 61:1587-1603.

Innami, S., Nakamura, A., Kato, K., Miyazaki, M., Nagayama, S. and Nishide, E. (1975) Polychlorinated biphenyls toxicity and nutrition IV. Fukuoka Acta Medica 66:579-584.

IPCS (1979) DDT and its derivatives. Environmental Health Criteria 9. World Health Organization, Geneva.

IPCS (1989c) Polychlorinated dibenzoparadioxins and dibenzofurans. World Health Organisation, Geneva.

IPCS (1989b) DDT and its derivatives – environmental aspects. Environmental Health Criteria. 83. World Health Organization. Geneva.

IPCS (1989a) Polychlorinated dibenzo-*para*-dioxins and dibenzofurans. Environmental Health Criteria. 88. World Health Organization. Geneva.

IPCS (1991) Lindane. Environmental Health Criteria. 124. World Health Organization. Geneva.

IPCS (1992) Diethylhexyl phthalate. Environmental Health Criteria 131. World Health Organization, Geneva.

IPCS (1993) Polychlorinated biphenyls and terphenyls (second edition). Environmental Health Criteria 140. World Health Organization, Geneva.

IPCS (1994a) Polybrominated Biphenyls. Environmental Health Criteria. 152. World Health Organization, Geneva.

IPCS (1994b) Brominated diphenyl ethers. Environmental Health Criteria 162. World Health Organization, Geneva.

IPCS (1996) Chlorinated paraffins. Environmental Health Criteria 181. World Health Organization, Geneva.

IRDC (1981a) 14-Day oral toxicity study in rats. Chlorinated paraffin: 58% chlorination of short chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-006.

IRDC (1981b) 14-Day oral toxicity study in rats. Chlorinated paraffin: 52% chlorination of long chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438005.

IRDC (1982) Teratology study in rats. Chlorinated paraffin: 58% chlorination of short chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-016.

IRDC (1983a) Teratology study in rabbits. Chlorinated paraffin: 70% chlorination of long chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-045.

IRDC (1983b) Teratology study in rats. Chlorinated paraffin: 43% chlorination of short length chain n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-015.

IRDC (1983c) Teratology study in rabbits. Chlorinated paraffin: 52% chlorination of intermediate chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-032.

IRDC (1984a) Teratology study in rats. Chlorinated paraffin: 70% chlorination of long chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan 438-045.

IRDC (1984b) Teratology study in rats. Chlorinated paraffin: 52% chlorination of intermediate chain length n-paraffins. International Research and Development Corporation. Mattawan, Michigan. 438-017.

Jansen, H.T., Cooke, P.S., Porcelli, J., Liu, T.C. and Hansen, L.G. (1993) Estrogenic and antiestrogenic actions of PCBs in the female rat: *in vitro* and *in vivo* studies. Reprod. Toxicol. 7:237-248.

Jefferies, D.J., French, M.C. and Osborne, B.E. (1971) The effect of *pp*'-DDT on the rate, amplitude and weight of the heart of the pigeon and Bengalese finch. Br Poult Sci. 12:387-399.

Jobling, S., Sheahan D., Osborne J.A., Matthiessen P. and Sumpter J.P. (1996) Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environ. Toxicol. Chem. 15:194-202.

Jones, M.K., Weisenburger, W.P., Sipes, I.G. and Russell, D.H. (1987) Circadian alterations in prolactin, corticosterone, and thyroid hormone levels and down-regulation of prolactin receptor activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 87:337-350.

Juarez de Ku, L.M., Sharma Stokkermans, M. and Meserve, L.A. (1994) Thyroxine normalizes polychlorinated biphenyl (PCB) dose-related depression of choline acetyltransferase (ChAT) activity in hippocampus and basal forebrain of 15-day-old rats. Toxicology. 94:19-30.

Kaiser, J. (1997) Synergy paper questioned at toxicology meeting. Science. 275: 1879.

Kaldas, R.S. and Hughes, C.L., Jr. (1989) Reproductive and general metabolic effects of phytoestrogens in mammals. Reprod Toxicol. 3:81-89.

Keil, J.E., Sandifer, S.H., Finklea, J.H. and Priester, L.E. (1972) Serum vitamin A elevation in DDT exposed volunteers. Bull Environ Contam Toxicol. 8:317-320.

Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A. and Wilson, E.M. (1995) Persistent DDT metabolite *p,p*'-DDE is a potent androgen receptor antagonist. Nature. 375:581-585.

KemI (1994) Risk assessment of polybrominated diphenyl ethers. The Swedish National Chemicals Inspectorate. Solna.

KemI (1997) Alkylphenols and alkylphenolethoxylates excluding nonylphenol. The Swedish Epa and the Swedish National Chemicals Inspectorate. Solna.

Keung, W.M. (1995) Dietary estrogenic isoflavones are potent inhibitors of beta-hydroxysteroid dehydrogenase of P. testosteronii. Biochem Biophys Res Commun. 215:1137-1144.

Kharat, I. and Saatcioglu, F. (1996) Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor. Cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. J. Biol. Chem., 271(18):10533-10537.

Kime, D.E. (1995) The effects of pollution on reproduction in fish. Rev. Fish Biol. Fish. 5:52-96.

Kimbrough, R.D. (1974) The toxicity of polychlorinated polycyclic compounds and related chemicals. Crit Rev Toxicol. 2:445-498.

Kimbrough, R.D. (1995) Polychlorinated biphenyls (PCBs) and human health: an update. Crit. Rev. Toxicol. 25:133-163.

Kociba, R.J., Keeler, P.A., Park, C.N. and Gehring, P.J. (1976). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35:553-574.

Korach, K.S., Sarver, P., Chae, K., McLachlan, J.A. and McKinney, J.D. (1988) Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. Mol. Pharmacol. 33:120-126.

Kovacevic, R., Vojinovic Miloradov, M., Teodorovic, I. and Andric, S. (1995) Effect of PCBs on androgen production by suspension of adult rat Leydig cells *in vitro*. J. Steroid. Biochem. Mol. Biol. 52:595-597.

Krishnan, V. and Safe, S. (1993). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. Toxicol. Appl. Pharmacol. 120:55-61.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L. and Feldman, D. (1993) Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. 132: 2279-2286.

Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S. and Gustafsson, J.A. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 138:863-870.

Lamb, J.C., Harris, M.W. McKinney, J.D. and Birnbaum, L.S. (1986) Effects of thyroid hormones on the induction of cleft palate by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6N mice. Toxicol. Appl. Pharmacol., 84: 115-124.

Lans, M.C., Klasson Wehler, E., Willemsen, M., Meussen, E., Safe, S. and Brouwer, A. (1993) Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and -dibenzofurans with human transthyretin. Chem. Biol. Interact. 88:7-21.

Lans, M.C., Spiertz, C., Brouwer, A. and Koeman, J.H. (1994) Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. Eur. J. Pharmacol. 270:129-136.

Laws, S.C., Carey, S.A., Hart, D.W. and Cooper, R.L. (1994) Lindane does not alter the estrogen receptor or the estrogen-dependent induction of progesterone receptors in sexually immature or ovariectomized adult rats. Toxicology. 92:127-142.

Leopold, A.S., Erwin, M. and Browning, B. (1976) Phytoestrogens: adverse effects on reproduction in Califonia quail. Science, 191:98-100.

Levin, E.D., Schantz, S.L. and Bowman, R.E. (1988) Delayed spatial alternation deficits resulting from perinatal PCB exposure in monkeys. Arch. Toxicol., 62:267-273.

Lilienthal, H. and Winneke, G. (1991) Sensitive periods for behavioral toxicity of polychlorinated biphenyls: determination by cross-fostering in rats. Fundam. Appl. Toxicol. 17:368-375.

Lindenau, A., Fischer, B., Seiler, P. and Beier, H.M. (1994) Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. Hum. Reprod. 9:772-780.

Link, R.P., Smith, J.C., Newton, D.I. (1958) Toxic effect of chlorinated naphthalenes in pigs. J. Am. Vet. Med. Assoc. 133: 83-85.

LoPresti, J.S. and Nicoloff, J.T. (1994) 3,5,3'-Triiodothyronine (T3) sulfate: a major metabolite in T3 metabolism in man. J. Clin. Endocrinol. Metab. 78:688-692.

Lu, Y., Wang, X. and Safe, S. (1994) Interaction of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin and retinoic acid in MCF-7 human breast cancer cells. Toxicol. Appl. Pharmacol. 127:1-8.

Lucier, G.W., Sonawane, B.R., McDaniel, O.S. and Hook, G.E. (1975) Postnatal stimulation of hepatic microsomal enzymes following administration of TCDD to pregnant rats. Chem. Biol. Interact. 11:15-26.

Lueprasitsakul, W., Alex, S., Fang, S. L., Pino, S., Irmscher, K., Kohrle, J. and Braverman, L.E. (1990) Flavonoid administration immediately displaces thyroxine (T4) from serum transthyretin, increases serum free T4, and decreases serum thyrotropin in the rat. Endocrinology. 126:2890-2895.

Lund, B.O., Örberg, J., Bergman, Å., Larsson, C., Bergman, A., Bäcklin, B.M., Håkansson, H., Madej, A., Brouwer, A. and Brunström, B. (1998) Cronic and reproductive toxicity of a mixture of 15 methylsulphonyl-PCBs and 3- methylsulphonyl-DDE in mink (*Mustela viso*n). Environ. Toxicol. Chem. (In press)

Lundholm, C. E. (1994) Changes in the levels of different ions in the eggshell gland lumen following *p,p*'-DDE-induced eggshell thinning in ducks. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 109:57-62.

Lundholm, C.E. (1995) Effects of methyl mercury at different dose regimes on eggshell formation and some biochemical characteristics of the eggshell gland mucosa of the domestic fowl. Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 110:23-28.

Lundkvist, U. (1990) Clinical and reproductive effects of Clophen A50 (PCB) administered during gestation on pregnant guinea pigs and their offspring. Toxicology, 6:249-257.

Mably, T.A., Bjerke, D.L., Moore, R.W., Gendron-Fitzpatrick, A. and Peterson, R.E. (1992b) *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3: Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharmacol., 114: 118-126.

Mably, T.A., Moore, R.W. and Peterson, R.E. (1992a) *In utero* and lactational ex-

posure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1: Effects on androgenic status. Toxicol. Appl. Pharmacol. 114:97-107.

Mably, T.A., Moore, R.W. Goy, R,W, and Peterson, R.E. (1992c) *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 2: Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. Toxicol. Appl. Pharmacol. 114:108-117.

MacLatchy, D.L. and Van Der Kraak, G.J. (1995) The phytoestrogen beta-sitosterol alters the reproductive endocrine status of goldfish. Toxicol. Appl. Pharmacol. 134:305-312.

MacLellan, K.N., Bird, D.M., Fry, D.M. and Cowles, J.L. (1996) Reproductive and morphological effects of o,p'-dicofol on two generations of captive American kestrels. Arch. Environ. Contam. Toxicol. 30:364-372.

Mann, A.H., Price, S.C., Mitchell, F.E., Grasso, P., Hinton, R.H. and Bridges, J.W. (1985) Comparison of the short-term effects of di(2-ethylhexyl) phthalate, di(n-hexyl) phthalate, and di(n-octyl) phthalate in rats. Toxicol. Appl. Pharmacol. 77:116-132.

Marchal Segault, D. (1982) Effets du lindane, insecticide organochlore, sur la thyroide des larves de Xenopus laevis Daudin en premetamorphose. C. R. Seances Soc. Biol. *Fil.* 176:839-848.

Mathieson, R.A. and Kitts, W.D. (1980) Binding of phyto-oestrogen and oestradiol-17 beta by cytoplasmic receptors in the pituitary gland and hypothalamus of the ewe. J Endocrinol. 85:317-325.

Matsuura, T. and Ross A.C. (1993) Regulation of Hepatic Lecithin: Retinol Acyltransferase Activity by Retinoic Acid. Arch Biochem Biophys. 301:221-227.

McKinney, J.D., Fawkes, J., Jordan, S., Chae, K., Oatley, S., Coleman, R.E. and Briner, W. (1985). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) as a potent and persistent thyroxine agonist: a mechanistic model for toxicity based on molecular reactivity. Environ. Health Perspect. 61:41-53.

McLachlan, J.A. (1997) Synergistic effects of environmental estrogens: Report withdrawn. Science 277:462-463.

Mellanen, P., Petanen, T., Lehtimaki, J., Makela, S., Bylund, G., Holmbom, B., Mannila, E., Oikari, A. and Santti, R. (1996) Wood-derived estrogens: studies *in vitro* with breast cancer cell lines and *in vivo* in trout. Toxicol. Appl. Pharmacol. 136: 381-388.

Miksicek, R.J. (1994). Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. J. Steroid. Biochem. Mol. Biol. 49:153-160.

Mirocha, C.J., Christensen, C.M. and Nelson, G.H. (1971) F-2 (zearalenone) estrogenic mycotoxin from Fusarium. In:(Kadis, S., Ciegler, A. and Ajl, S.J. eds.), Academic Press, New York. Microbial Toxins, 7:107-138.

Moon, R.C., Mehta, R.G. and Detrisac, C.J. (1992) Retinoids as chemopreventive agents for breast cancer. Cancer Detect. Prev. 16:73-79.

Moore, R.W., Potter, C.L., Theobald, H.M., Robinson, J.A. and Peterson, R.E. (1985). Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 79:99-111.

Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C. and Kimmel, C.A. (1987) The developmental toxicity of bisphenol A in rats and mice. Fundam Appl. Toxicol. 8:571-582.

Morrissey, R.E., Lamb, J.C., Morris, R.W., Chapin, R.E., Gulati, D.K. and Heindel, J.J. (1989) Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fund. Appl. Toxicol. 13:747-777.

Morse, D.C. and Brouwer, A. (1995) Fetal, neonatal, and long-term alterations in hepatic retinoid levels following maternal polychlorinated biphenyl exposure in rats. Toxicol. Appl. Pharmacol. 131: 175-182.

Morse, D.C., Plug, A., Wesseling, W., van den Berg, K.J. and Brouwer, A. (1996) Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol. Appl. Pharmacol. 139:252-261.

Mousavi, Y. and Adlercreutz, H. (1993) Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. Steroids. 58:301-304.

Muller, W.F., Hobson, W., Fuller, G.B., Knauf, W., Coulston, F. and Korte, F. (1978) Endocrine effects of chlorinated hydrocarbons in rhesus monkeys. Ecotoxicol Environ Safety. 2:161-172.

Murray, F.J., Smith, F.A., Nitschke, K.D., Humiston, C.G., Kociba, R.J. and Schwetz, B.A. (1979) Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50: 241-252.

Naturvårdsverket (1989) Livsmedelstoxikologiska aspekter på rötslam i jordbruket. Report No. 3623.

Nelson, J.A. (1974) Effects of dichlorodiphenyltrichloroethane (DDT) analogs and polychlorinated biphenyl (PCB) mixtures on 17beta-(3H)estradiol binding to rat uterine receptor. Biochem Pharmacol. 23:447-451.

Ness, D.K., Schantz, S.L., Moshtaghian, J. and Hansen, L.G. (1993) Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol. Lett. 68:311-323.

Nhachi, C.F. and Kasilo, O.J. (1990) Occupational exposure to DDT among mosquito control sprayers. Bull. Environ. Contam. Toxicol. 45:189-192.

Nilsson, C., Hanberg, A., Trossvik, C., Manzoor, E. and Håkansson, H. (1996) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin affects retinol esterification in rat hepatic stellate cells and kidneys. Environ Toxicol Pharmacol 2:17-23.

Nord (1992) Risk assessment of polychlorinated biphenyls. Nordisk Ministerråd. Köpenhamn. 26.

NTP (1982) Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for possible carcinogenicity (gavage study). U.S. DHHS, PHS. Research Triangle Park, NC, USA. 201.

NTP (1985) Di-n-octylphthalate: Reproductive and fertility assessment in CD-1 mice when administered in feed. National Institute of Environmental Health Sciences, National Toxicology Program. Research Triangle Park, NC.

Oishi, S. and Hiraga, K. (1980) Testicular atrophy by phthalic acid esters: Effect on testosterone and zinc concentrations. Toxicol. Appl. Pharmacol. 53:35-41.

Olafson, P. (1947) Hyperkeratosis (X disease) of cattle. Cornell Veterinarian 37: 279-291.

Olson, J.A. (1969) Recent developments in the fat-soluble vitamins. Metabolism and function of vitamin A. Federation Proceedings 28:1670-1677.

Overmann, S.R., Kostas, J., Wilson, L.R. Shain, W. and Bush, B. (1987) Neurobehavioral and somatic effects of perinatal PCB exposure in rats. Environ. Res., 44:56-70.

Örberg, J., Johansson, N., Kihlström, J.E. and Lundberg, C. (1972) Administration of DDT and PCB prolongs oestrous cycle in mice. Ambio, 1:148-149.

Örberg, J. and Kihlstrom, J. E. (1973) Effects of long-term feeding of polychlorinated biphenyls (PCB, Clophen A

60) on the length of the oestrous cycle and on the frequency of implanted ova in the mouse. Environ. Res. 6:176-179.

Patnode, K.A. and Curtis, L.R. (1994) 2,2',4,4',5,5'- and 3,3',4,4',5,5'-hexachlorobiphenyl alteration of uterine progesterone and estrogen receptors coincides with embryotoxicity in mink (*Mustela vision*). Toxicol. Appl. Pharmacol. 127:9-18.

Peakall, D.B. (1974) Effects of Di-n-butyl and di-2-ethylhexyl phthalate on the eggs of ring doves. Bull Environ Contam Toxicol. 12:698-670.

Peterson, R.E., Theobald, H.M. and Kimmel, G.L. (1993) Developmental an d reproductive toxicity of dioxins and related compounds: cross-species comparisons. Crit. Rev. Toxicol., 23(3):283-335.

Phillips, W.E.J. (1963) DDT and the metabolism of vitamin A and carotene in the rat. Can. J. Biochem. Physiol. 41:1793-1802.

Phillips, W.E.J., Hatina, G., Villeneuve, D.C. and Grant, D.L. (1971) Multigeneration studies on the effect of dietary DDT on the vitamin A status of the weanling rat. Canadian Journal of Physiology and Pharmacology 49: 382-386.

Piersma, A.H., Verhoef, A. and Dortant, P.M. (1995) Evaluation of the OECD 421 reproductive toxicity screening test protocol using butyl benzyl phthalate. Toxicology 99:191-197.

Pohjanvirta, R., Håkansson, H., Juvonen, R. and Tuomisto, J. (1990) Effects of TCDD on vitamin A status and liver microsomal enzyme activities in a TCDD-susceptible and a TCDD-resistant rat strain. Food Chem Toxicol.28:197-203.

Poon, R., LeCavalier, P., Chan, P., Viau, C., Håkansson, H., Chu, I. and Valli, V.E. (1995) Subchronic toxicity of medium-chain chlorinated paraffin in the rat. J. Appl. Toxicol. 15:455-463.

Potter, C.L., Moore, R.W., Inhorn, S.L., Hagen, T.C. and Peterson, R.E. (1986) Thyroid status and thermogenesis in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 84:45-55.

Potter, C.L., Sipes, I.G. and Russell, D.H. (1983) Hypothyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administration. Toxicol. Appl. Pharmacol. 69:89-95.

Prasad, A.K., Pant, N., Srivastava, S.C., Kumar, R. and Srivastava, S.P. (1995) Effect of dermal application of hexachlorocyclohexane (HCH) on male reproductive system of rat. Hum. Exp. Toxicol. 14:484-488.

Potter, S.M., Pertile, J. and Berber-Jimenez, M.D. (1996) Soy protein concentrate and isolated soy protein similarly lower blood serum cholesterol but differently affect thyroid hormones in hamsters. Nutr. Met. 2007-2011.

Pudelkiewics, W.J., Boucher, R.V., Callenbach, E.W. and Miller, R.C. (1959) Some physiological responses of New Hampshire chickens to a mixture of penta- and hexachloronaphthalenes. Poultry Sci. 38:424-430.

van Raaij, J.A., van den Berg, K.J. and Notten, W.R. (1991) Hexachlorobenzene and its metabolites pentachlorophenol and tetrachlorohydroquinone: interaction with thyroxine binding sites of rat thyroid hormone carriers *ex vivo* and *in vitro*. Toxicol. Lett. 59: 101-107.

Recupero, A.R., Coleoni, A.H., Cherubini, O. and Oviedo, A. (1983) Selective alterations in hepatic nuclear T3-receptors and enzyme responses by glucocorticoid deficit or excess. Acta. Endocrinol. Copenh. 104:485-489.

Roberts, E.S., Vaz, A.D.N. and Coon, M.J. (1992) Role of isozymes of rabbit microsomal cytochrome P-450 in the metabolism of retinoic acid, retinol, and retinal. Mol. Pharmacol. 41:427-433.

vom Saal, F.S., Nagel, S.C., Palanza, P., Boechler, M., Parmigiani,S. and Welshons, W.V. (1995) Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behaviour in male mice. Toxicol. Lett. 77:343-350.

Safe, S. and Krishnan, V. (1995) Chlorinated hydrocarbons: estrogens and antiestrogens. Toxicol. Lett. 82-83:731-736.

Sager, D.B. and Girard, D.M. (1994) Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. Environ. Res. 66:52-76.

Scarlata, S. and Miksicek, R. (1995) Binding properties of coumestrol to expressed human estrogen receptor. Mol. Cell. Endocrinol. 115:65-72.

Scott, M.L., Zimmermann, J.R., Marinsky, S. and Mullenhoff, P.A. (1975) Effects of PCBs, DDT, and mercury compounds upon eff production, hatchability and shell quality in chickens and Japanese quail. Poultry Science. 54:350-368.

Seidler, H., Hartig, M. and Engst, R. (1976) [Effects of DDT and lindane on thyroid function in the rat]. Nahrung. 20:399-406.

Seo, B.W., Li, M.H., Hansen, L.G., Moore, R.W., Peterson, R.E. and Schantz, S.L. (1995) Effects of gestational and lactational exposure to coplanar polychlorinated biphenyl (PCB) congeners or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thyroid hormone concentrations in weanling rats. Toxicol. Lett. 78:253-262.

Serraino, M. and Thompson, L.U. (1991) The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett. 60:135-142.

Serrone, D.M., Birtley, R.D., Weigand, W. and Millisher, R. (1987) Toxicology of chlorinated paraffins. Food Chem. Toxicol. 25:553-562.

Setchell, K.D.R. and Adlercreutz, H. (1988) Mammalian ilgnans and phytoestrogens: recent studies on their formation, metabolism and biological role in health and disease. In: The role of gut microflora in toxicity and cancer. (Rowland, I.A. ed.) Academic Press, New York. pp. 315-345.

Sewall, C.H., Flagler, N., Vanden Heuvel, J.P., Clark, G.C., Tritscher, A.M., Maronpot, R.M. and Lucier, G.W. (1995) Alterations in thyroid function in female Sprague-Dawley rats following chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 132:237-244.

Sharpe, R.M., Fisher, J.S., Millar, M. M., Jobling, S. and Sumpter, J.P. (1995) Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. Environ. Health Perspect. 103.

Singh, S. and Singh, T.P. (1987) Evaluation of toxicity limit and sex hormone production in response to cythion and BHC in the vitellogenic catfish *Clarias batrachus*. Environ. Res. 42:482-488.

Sircar, S. and Lahiri, P. (1989) Lindane (gamma-HCH) causes reproductive failure and fetotoxicity in mice. Toxicology. 59:171-177.

Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N., Olea Serrano, F. (1995) The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ. Health Perspect. 103 (suppl 7):113-122.

Soto, A.M., Justicia, H, Wray, J. W. and Sonnenschein, C. (1991) *p*-Nonyl- phenol: an estrogenic xenobiotic released from “modified” polystyrene. Environ. Health Perspect. 92:167-173.

Steinmetz, R., Young, P.C.M., Caperell-Grant, A., Gize, E.A., Madhukar, B.V., Ben-Jonathan, N. and Bigsby, R.M. (1996) Novel estrogenic action of the pesticide residue ß-hexachlorocyclohexane in human breast cancer cells. Cancer Reserarch, 56:5403-5409.

TemaNord 1996:580, (1996) Chemical with estrogen-like effects. Nordic Council of ministers, Copenhagen 1996. ISBN 92-9120-918-X.

Thunberg, T., Ahlborg, U.G., Johnsson, H. (1979) Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8 tetrachlorodibenzo-*p*-dioxin. Arch Toxicol 42:265-274.

Tinsley, I.J. (1969) DDT effect on rats raised on alpha-protein rations: Growth and storage of liver vitamin A. J. Nutr. 98:319-324.

Underwood, B.A. (1984) Sporn M.B., Roberts A.B., Goodman, D.S. (eds)The Retinoids Volume 1. Academic Press, Inc. Orlando, USA, pp 281-392.

US/EPA (1985) Health assessment document for polychlorinated dibenzo-*p*-dioxins. U.S. Environmental Protection Agency. Cincinnati, OH.

US/EPA (1990) Drinking water criteria document for polychlorinated biphenyls (PCBs). U.S. Environmental Protection Agency. Cincinnati, OH.

Vavrova, M., Mikulik, A. and Dobes, M. (1976) Chlorovane uhlovodiky v potravinach zivocisneho puvodu a jejich vliv na vlastnosti masa a tuku drubeze. Vet. Med. Praha. 21:187-93.

Vecchini, F., Lenoir-Viale, M.C., Cathelineau, C., Magdalou, J., Bernard, B.A. and Shroot B (1994) Presence of a retinoid responsive element in the promoter region of the human cytochrome P4501A1 gene. Biochem. Biophys. Res. Commun. 201:1205-1212.

Vecchini, F., Mace, K., Magdalou, J., Mahe, Y., Bernard, B.A., and Shroot, B. (1995) Constitutive and inducible expression of drug metabolizing enzymes in cultured human keratinocytes. Br. J. Dermatol. 132:14-21.

Vonier, P.M., Crain, D.A., McLachlan, J.A., Guillette, L.J., Jr. and Arnold, S.F. (1996) Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environ. Health Perspect. 104:1318-1322.

Weston, W.M., Nugent, P. and Greene, R.M. (1995) Inhibition of retinoic-acid-induced gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem Biophys Res Commun 207:690-694.

White, R., Jobling, S., Hoare, S.A., Sumpter, J.P. and Parker, M.G. (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135:15-182.

Whitlock J.P., Jr. (1993) Mechanistic aspects of dioxin action. Chem. Res. Toxicol. 6:754-763.

Waern, F. (1997) Alkylphenols and alkylphenolethoxylates excluding nonylphenol. Manuscript prepared for the Swedish EPA and the Swedish National Chemicals Inspectorate.

Wang, T.T., Sathyamoorthy, N. and Phang, J.M. (1996) Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis. 17:271-275.

Welch, R.M., Levin, W. and Conney, A.H. (1969) Estrogenic action of DDT and its analogs. Toxicol. Appl. Pharmacol. 14: 358-367.

Wolf, G. (1984) Multiple functions of vitamin A. Physiol Rev 64:873-938.

Wyatt, I., Coutts, C.T. and Elcombe, C.R. (1993) The effect of chlorinated paraffins on hepatic enzymes and thyroid hormones. Toxicology. 77:81-90.

Yadav, A.K. and Singh, T.P. (1986) Effect of pesticide on circulating thyroid hormone levels in the freshwater catfish, *Heteropneustes fossilis* (Bloch). Environ. Res. 39:136-142.

Zile, M.H. (1992) Vitamin A homeostasis endangered by environmental pollutants. Proc. Soc. Exp. Biol. Med. 201:141-153.

Zile, M.H., Bank, P.A., Roltsch, I.A. (1989) Alterations in vitamin A metabolism by polyhalogenated aromatic hydrocarbons. Z. Ernahrungswiss. 28:93-102.

7. General conclusions and recommendations

It has been known for several decades that environmental contaminants may disturb endocrine function. More recently it has been demonstrated that pollutants can interact with hormone systems via a number of different mechanisms. There has been a focus on estrogenic environmental contaminants in the current discussion about endocrine disrupters. This may partly be due to the availability of sensitive *in vitro* tests with specific endpoints for estrogen receptor agonists. However, interaction of environmental compounds with other steroid hormones, thyroid hormones and retinoids may be as widespread and important for impaired reproduction and development as the estrogenic effects.

It is clear that many different pollutants can interfere with all the hormone systems discussed in this report, but it is uncertain to which extent humans and wildlife are exposed to levels high enough to cause adverse health effects. Effects on reproduction and endocrine function have been observed in wildlife in different areas contaminated with anthropogenic compounds. In Sweden, disturbed reproduction has been reported in fish, predatory birds and fish-eating mammals. The documented effects in wildlife have in most cases been correlated to well-known contaminants such as DDT and PCB or to contaminants present in effluents from the pulp and paper industry. In spite of a downward trend for most persistent organic pollutants in Swedish wildlife, levels of PCBs in certain specimens of fish-eating mammals are still higher than those known to impair reproduction in the mink. The salmon in the Baltic have been showing increased incidence of fry mortality (M74). This disease has been linked to thiamine deficiency and there is little evidence at present that M74 is related to xenobiotics.

Various developmental and reproductive disorders in humans have been proposed to be due to environmental pollutants. Epidemiological data from several independent studies indicate that fetal and/or early postnatal exposure to persistent organochlorines could be associated with disturbed neural development, decreased birth weight, and possibly with altered thyroid hormone levels. These data are supported by experimental findings in several species. Available data do not indicate a risk for adverse effects in the general Swedish population. However, the safety margins for foetuses/neonates may be narrow and the exposure within the population varies depending on dietary habits. Furthermore, the normal functions and interrelationships of the various hormone systems, and their connections with the nervous and immune systems, are incompletely known and contaminants may have subtle effects not yet identified. There are also potential EDSs present in the environment that have not been thoroughly evaluated for adverse effects in whole animal systems

and human exposure to most environmental contaminants is incompletely known. In Sweden there seems to be an increasing trend in testicular cancers and hypospadias but there is no obvious relationship between exposure to environmental contaminants and these disorders.

GENERAL RECOMMENDATIONS FOR FUTURE RESEARCH

Recommended areas for future research activities include:

- Development of *in vitro* and *in vivo* models for endocrine disruption.
- Experimental effect studies and determination of dose-response relationships.
- Chemical identification of EDSs present in the environment and determination of human and wildlife exposure.
- Identification and monitoring of endocrine disruption in wildlife and humans.
- Basic research about hormone systems and elucidation of the mechanisms of action of endocrine disrupters.
- Development of methods to assess the risk of EDSs.

Development of test methods

Effects of chemicals have to be evaluated using various *in vitro* and *in vivo* test systems. Modern techniques such as molecular modelling and transfection of cultured cells with cloned receptor genes may be used for rapid screening purposes but cannot replace *in vivo* studies.

RECOMMENDATIONS

- *In vitro* tests for screening should be further developed and validated.
- Test methods using whole organisms should be developed and used in testing of potential EDSs.
- Sensitive life-stages and endpoints have to be identified.
- QSAR models may be used to give priority to compounds for further testing with the more labour-consuming and expensive *in vitro* and *in vivo* tests.

Effect studies in experimental animals

Both new and existing chemicals need to be characterized for endocrine-disrupting effects. Various *in vitro* tests will generate a great amount of data aiding in the identification of potential EDSs. The potencies of these potential EDSs to cause adverse health effects have to be determined in whole animal models.

RECOMMENDATIONS

- Dose-response relationships should be determined and threshold levels identified.
- The kinetics of potential EDSs and effects of metabolites need to be considered.
- Additive, synergistic and antagonistic interactions between chemicals in mixtures have to be examined using both *in vitro* and whole animal systems.

Chemical identification of EDSs

There is insufficient information about the extent to which humans and wildlife are exposed to EDSs. For most chemicals there is also a lack of data on geographical differences and temporal trends in concentration levels.

RECOMMENDATIONS

- Non-discriminating analysis should be performed in order to obtain information on exposure.
- Internal exposure in humans and top predators has to be determined.
- Analytical methods for less persistent compounds and their metabolites need to be developed.
- Potential EDSs should be synthesized for use in experimental studies and as reference substances.

Wildlife studies

In wildlife, adverse health effects have been observed and certain populations and specimens are probably exposed to contaminant levels high enough to cause reproductive impairment. There are large species differences in sensitivity to environmental contaminants and animals may be particularily vulnerable to EDSs at specific life stages or developmental periods.

RECOMMENDATIONS

- Sensitive and specific biomarkers for endocrine disruption should be identified.
- Various species and developmental stages have to be studied.
- Sentinel species should be identified and biomarkers included in monitoring programmes.

Human epidemiology

Data from epidemiological studies suggest that environmental pollutants may interfere with foetal and neonatal development at relevant exposure levels. There are indications that certain genital malformations and endocrine-related cancer forms in re-

productive organs are increasing, and a decrease in sperm quality has been suggested. Any causal relationship between exposure to environmental contaminants and these disorders has yet to be demonstrated.

RECOMMENDATIONS

- Exposure assessments need to be improved. Studies should include internal and external exposure data.
- Frequencies of endocrine-related reproductive and developmental disorders should be compared geographically and temporaly between populations differently exposed to contaminants.
- A combination of cohort studies of especially exposed populations and case reference studies are needed.
- Examples of health outcomes to be studied are birth weight, growth during infancy, neural development, time to pregnancy, sperm quality, incidence of hypospadias, hormone levels, and hormone-related neoplasms.
- Methods have to be standardized in order to enable comparisons between different studies.

Basic research and elucidation of mechanisms

For many of the observed adverse effects on endocrine systems, the mechanisms are not understood. Often, it is not clear whether the effects are caused by direct interference with the endocrine systems or if they are secondary to effects on other systems.

RECOMMENDATIONS

- Receptor assays, cells containing reporter systems, and other *in vitro* systems are important tools and should be used for studying the mechanisms of action of EDSs.
- Basic research on normal functions of hormonal systems is needed. Such studies should be combined with studies elucidating the mechanisms involved in the interference of EDSs with these systems.
- Research on normal and chemically disturbed reproductive and developmental processes should be carried out in various species.
- Studies of hormone action during embryogenesis and the effects of chemical interferences during this period are needed.

Methods for risk assessment of EDSs

RECOMMENDATIONS

- New and improved methods are required for comparison and ranking of EDSs within and between compound classes exhibiting the same type of hormone disrupting effect.
- There is also a need for new and improved methods to compare and rankmixtures of EDSs; both EDSs exhibiting the same and different types of hormone disrupting effects.
- Systematic evaluation and comparison of effect data taking chemical and physical properties of EDSs into consideration is needed.

RESEARCH TO BE SUPPORTED BY A SWEDISH PROGRAMME

- The Swedish EPA should support research in most of the areas suggested above.
- Development of *in vitro* and *in vivo* test models for EDSs should be supported as a contribution to the OECD test guideline program. The development of *in vivo* models also constitutes a basis for identification of suitable biomarkers to be used in biomonitoring in the Swedish environment. Thus the integration of laboratory and field studies should be supported.
- Endocrine disrupting effects of effluents from the pulp, paper and chemical industries as well as from sewage treatment plants in Sweden should be surveyed. The extent of adverse effects in recipient water bodies should be determined and any active compounds identified.
- Extended assessment of the exposure of Swedish wildlife to EDSs is eeded. Thus new analytical techniques need to be developed and applied. The exposure of top predators such as Baltic seals and harbour porpoises, otters, and predatory birds to persistent organic pollutants should be monitored and adverse effects in these species identified. Fish are continuously exposed to both persistent organic pollutants and non-persistent compounds and due to their labile sex determination they may constitute a highly sensitive group. The effects of these compounds on fish should therefore be monitored using stationary species such as eelpout and perch, which are present in Swedish waters. Results from experimental studies and determinations of dose-response relationships for various compounds are crucial for the selection of compounds to be included in extended monitoring activities.
- Exposure assessment of the Swedish human population to EDSs should be improved and constitute a basis for epidemiological studies.
- Research on compounds to which wildlife and humans are significantly exposed,

and which are still being spread, should have priority. For instance, brominated flame retardants such as PBDEs and tetrabromobisphenol A are still in use, are detected in biota, and insight into their distribution in the environment and possible endocrine disrupting effects are limited.

- The development and evaluation of novel tools to estimate health and environmental risk associated with exposure to EDSs should be supported.
- Although basic knowledge of hormonal systems and reproductive and developmental processes needs to be extended, research in these areas should mainly be supported by other sources. However, research on the various mechanisms of action of EDSs should be supported.

8. ABBREVIATIONS

AF-1	Activation function domain 1 in receptor
AF-2	Activation function domain 2 in receptor
Ah-R	Aryl hydrocarbon receptor (dioxin receptor)
AP-1	Activator protein 1 (cJun and cFos dimers)
APE	Alkylphenol ethoxylate
Araclor	PCB mixture
ARAT	Acyl coenzyme A retinol acyl transferase
BDE	See PBDE
BBP	Butylbenzyl phthalate
BCF	Bioconcentration factor
BCPS	Bis-(4-chlorophenyl)sulphone
CB	See PCB
CDE	See PCDE
Clophen	PCB mixture
CN	See PCN
CP	Chlorinated paraffin
CRABP	Cellular retinoic acid binding protein
CRBP	Cellular retinoid binding protein
CS	See OCS
CYP	Cytochrome P450
DBP	Dibutyl phtalate
DDT	Dichloro diphenyl trichloroethane [p,p-DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane]
DEHP	Di(2-ethylhexyl)phtlalate
DES	Diethylstilbestrol
DIT	Diiodotyrosyl
E2	Estrogen
EMS	Early mortality syndrome
ER	Estrogen receptor
ERE	Estrogen receptor element (enhancer region in gene)
EROD	Ethoxyresorufin O-deethylase
FSH	Follicle stimulating hormone
GABA	Gamma-aminobutyric-acid
GAD	Glutamic acid decarboxylase
GLEMEDS	Great Lakes embryo mortality, edema and deformties syndrome
HCB	Hexachlorobenzene
hCG	Human corionnic gonadotropin
HCH	Lindane

17 HSD	17 hydroxysteroid dehydrogenase
Kow	Partition koefficient between oktanol and water
LH	Luteinizing hormone
LOAEL	Lowest observable adverse efffect level
LRAT	Lecitin retinol acyl transferase
M74	Mortality 1974 (see also EMS)
MCF-7	Human breast cancer cell line
MDR	Multi drug resistance
MIH	Müllerian inhibitory hormone
MIT	Monoiodotyrosyl
MSF-PCB	Methyl sulphonyl metabolite of PCB
NOAEL	No observable adverse effect level
NP	Nonylphenol
OCS	Octachlorostyrene
OH-PCB	Hydroxylated metabolites of PCB
OP	Oktylphenol
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDE	Polychlorinated diphenylethers
PCDF	Polychlorinated dibenzofuran
PCN	Polychlorinated naphthalene
PCP	Pentachlorophenol
RAR	Retionic acid receptor
RARE	Retinoic acid response elements(enhancer region in gene)
RBP	Retinoid binding protein
REH	Retinylester hydrolase
RRE	Raloxifene response element (suggested)
RU486	Antiprogestin
RXR	Retionid X receptor
SHBG	Steroid hormone binding globulin
TBBP-A	Tetrabromobisphenol A
TBG	Thryoxine-binding globulin
TBT	Tributyltin
TCDD	Tetrachloro dibenzo-*p*-dioxin
TCPM	Tris (4-chlorophenyl)methane
TGF-β3	Transforming growth factor-β3
T3	Thiiodothyronine
T4	Thyroxine
TTR	Transthyretin