A variety of human health concerns have been raised in relation to endocrine disruptors. Attention has focused on health end points considered to be potentially at risk because either their development or their later functioning in adult life is known or thought to be influenced by exposure to chemicals with endocrine activity.

In this chapter, four main areas are reviewed: reproduction, neurobehavior, immune function, and cancer. The order of the chapter sections reflects the continuum of life from conception through adult life. Because sex hormones and thyroid hormones are major determinants of development and function of the reproductive, central nervous, and immune systems, much of the experimental research to date has focused on the effects of EDCs on these key hormone systems and their target tissues, and possible links to human health effects. Endocrine-mediated mechanisms are well established for certain human cancers, and these are also addressed.

Although a few examples in this chapter clearly demonstrate adverse effects in humans after high exposures to environmental chemicals (such as poisoning incidents), the data are much less clear for lower levels of exposure, either to single chemicals or to mixtures with potentially similar actions. It is these lower levels of exposure that raise the most important public health questions, because even small shifts in population distributions of adverse health outcomes, such as infertility or lowered IQ, could potentially have considerable impact on the overall health of large populations. The inherent problems in comparing human, laboratory, and epidemiological studies conducted at different times and locations and under different conditions continue to affect our ability to draw firm conclusions about the existence of any global disease trends, and the lack of adequate exposure data severely hampers the exploration of hypotheses regarding possible causes of any identified trends. Similarly, the lack of exposure data during critical periods of development that influence later functioning in life make it difficult to draw causative associations between exposure and effect.

Hence, this chapter not only explores the available human health data but also draws on relevant data from experimental studies in laboratory animals where they support or cast doubt on the biological plausibility of an adverse effect on human health from EDCs. This chapter is not intended to be a comprehensive review of the experimental literature, which has grown exponentially in the last decade. However, it is only by integrated consideration of the background endocrinology, the human data, and the animal findings that the strength of the endocrine disruptor hypothesis can be evaluated.

5.1 Reproduction

5.1.1 Introduction

The possibility that environmental exposure to chemicals might affect human reproduction is not new. However, the hypothesis that environmental chemicals acting as EDCs could be causative agents of changes in population-based, reproductive health trends is relatively recent. Changes occurring in various human reproductive health statistics, particularly changes in temporal and geographical trends, play a key role in the debate about possible effects of exposure to EDCs. Although the main focus to date has been on male reproductive health (Toppari et al., 1996), this review covers both male and female reproductive function.

Much of the interest in the male reproductive system has stemmed from an hypothesis proposed by Sharpe and Skakkebaek (1993) that agents that interfere with normal development of the reproductive system via an endocrine mechanism could plausibly be related to increases noted in human male reproductive disorders over a number of years. In particular, a link was made between developmental events that could result in decreased sperm count/quality and increased incidences of testicular cancer, testicular maldescent (cryptorchidism), and male reproductive tract malformations, such as hypospadias. These could be expressions of one underlying entity, the testicular dysgenesis syndrome (Skakkebaek et al., 2001), which could result from disruption of gonadal development during fetal life. Although the evidence linking such effects to human exposure to chemicals is very weak or nonexistent, treatment of experimental animals with certain chemicals during critical windows of development of the male reproductive system can result in deficits of the type proposed by the hypothesis (Chapter 3, section 3.12). The one major exception is the production of testicular cancer (section 5.4.4). Seminoma, the major

List of Abbreviations

2,4-D 2,4-Dichlorophenoxyacetic acid
AhR Aryl hydrocarbon receptor
AR Androgen receptor
CI Confidence interval
CIS Carcinoma *in situ*
d day
DBCP Dibromodichloropropane
DDE Dichlorodiphenyl dichloroethylene
DES Diethylstilbestrol
DDT Dichlorodiphenyl trichloroethane
DHT Dihydrotestosterone
DTH Delayed-type hypersensitivity
E_2 17β-Estradiol
EDCs Endocrine-disrupting chemicals
EGCG (–)-Epigallocatechin gallate
EGF Epidermal growth factor
ER Estrogen receptor (α and β isoforms)
FSH Follicle-stimulating hormone
GD Gestational day
GR Glucocorticoid receptor
HCB Hexachlorobenzene
HPOA Hypothalamic preoptic area
HRT Hormone replacement therapy
IARC International Agency for Research on Cancer
Ig Immunoglobulin
IPCS International Programme on Chemical Safety
LA Los Angeles
LH Luteinizing hormone
LOAEL Lowest observed adverse effect level
MRC Medical Research Council
NOAEL No observed adverse effect level
NYC New York City
OECD Organisation for Economic Co-operation and Development
OR Odds ratio
PBBs Polybrominated biphenyls
PCBs Polychlorinated biphenyls
PCDDs Polychlorinated dibenzodioxins
PCOS Polycystic ovary syndrome
PCDFs Polychlorinated dibenzofurans
PHAHs Polyhalogenated aromatic hydrocarbons
PND Postnatal day
RACB Reproductive assessment through continuous breeding
T_3 Triiodothyronine
T_4 Thyroxine
TCDD 2,3,7,8-Tetrachlorodibenzyl-*p*-dioxin
TEQs Toxic equivalent quotients
TGF Tumor growth factor
TPOs Thyroid peroxidase inhibitors
TSH Thyroid-stimulating hormone
USA United States of America
US EPA United States Environmental Protection Agency
US FDA United States Food and Drug Administration
wt weight

form of human testicular cancer, is extremely rare in laboratory animals, and until recently, no experimental model existed for its production (section 5.4.4.2). However, some chemicals have been shown to produce interstitial (Leydig) cell tumors, particularly in rodents, that have been related to disturbances in normal endocrine control of male reproductive function.

Changing trends in female reproductive health have been much less studied than those in males. However, female reproductive development is also susceptible to endocrine interference (Chapter 3), and effects on female reproductive tissues and pregnancy outcomes may be of value as indicators of environmental influences. A number of aspects of female reproductive health are discussed below, and breast cancer is discussed in section 5.4.2.

The following sections summarize the available literature on adverse outcomes in human reproductive health that have either been proposed to be due to exposure to endocrine disruptors, or for which an endocrine mechanism is plausible or has been demonstrated. A number of examples of chemical influences on human reproduction are cited, but it should be noted that evidence that these are clearly linked to endocrine-mediated effects is lacking. Emphasis has been put on the plausibility of the endocrine disruptor hypothesis in relation to each end point considered and on causality. Although the focus is on the human literature, experimental animal data have been included to explore plausibility issues and potential mechanistic pathways involving endocrine disruption.

5.1.2 Sperm Quality and Testis Function

5.1.2.1 Assessment of testis function. Spermatogenesis is the process whereby normal spermatozoa are produced from spermatogonia in the seminiferous epithelium of the testis. Initial mitotic divisions result in proliferation of the spermatogonial stem cells that will eventually enter meiotic prophase, where, as spermatocytes, the cells undergo a series of transformations and eventually reduction divisions to reduce the chromosome number by half and produce spermatids. These round cells then undergo a metamorphosis to produce the elongated spermatids that are eventually released from the epithelium to enter the lumen of the seminiferous tubule, thence to the rete testis, the efferent ducts, and epididymis, where the sperm acquire the ability to become motile and fertilize an ovum. All of these cellular changes are under close hormonal control at the endocrine, paracrine, and autocrine levels (Chapter 3) and thus are potential targets for the effects of endocrine-active chemicals, including (anti)estrogens (anti)androgens, steroid biosynthesis inhibitors, and a variety of growth factor mimics.

In animal species, all of these potential targets can be evaluated in standard bioassays and research study designs following treatment with chemicals, and clear relationships can be developed between internal target dose and tissue histology, cellular function, and biochemical end points. The ability to undertake such studies in human males is far more limited and is normally restricted to circulating hormone levels (which tend to be insensitive or reflect only serious deficits) or some measure of sperm quality (number, motility, morphology, function). Although these parameters can be measured in animals to enable a direct comparison with humans, the process for the production of sperm is far more efficient in laboratory animal species, and small perturbations have a lesser chance to induce functional deficits.

Potential fertility in the male is reduced when the sperm concentration is very low. However, there is no consensus on the value of this limit. From studies in couples attempting to conceive, it has been found that the fertility potential of men decreased when the sperm concentration was under 40×10^6/ml (Bonde et al., 1998). A similar figure of 48×10^6/ml has also been recently reported (Guzick et al., 2001).

Other sperm characteristics, such as the percentage of motile forms or the incidence and the type of morphological abnormalities, seem to be more directly related to male fertility potential. Recent data that attempt to define a "normal" semen evaluation indicate, for example, that sperm morphology is a better discriminator between fertile and infertile men than is sperm concentration (Guzick et al., 2001). Sperm morphology and motility could also be useful markers of toxic damage even in the absence of any effect on fertility. The fertilizing ability of spermatozoa is a complex phenomenon involving many molecular and cellular events. Some of them are essential, such as the kinetic function allowing the sperm to reach the oocyte, or capacitation and the acrosome reaction necessary for the interaction with the zona pellucida and the fusion with the oocyte plasma membrane. The nuclear composition and DNA integrity are also important factors for normal embryo development. Sophisticated biological tests have been developed in recent years to evaluate various sperm functions, but no single test can ideally measure the fertility potential of a man.

In practice, if the sperm count is used to compare fertility potential of different populations at different times or in different places, it may be more appropriate to analyze the distribution of sperm concentration and more especially the proportion of men with lower values instead of comparing the arithmetic means or even the medians or the geometric means. Sperm concentration is dependent on the volume of seminal plasma in that the germinal cells are diluted at ejaculation. Because the seminal plasma volume may vary, sperm count (concentration × volume) rather than sperm concentration may be a better quantitative marker of spermatogenesis, although both are used clinically. It is also useful to compare the percentage of motile and morphologically abnormal sperm. Alternatively, the concentration of motile sperm, as calculated by Irvine et al. (1996), may better reflect the fertility potential than does the sperm count. Although sperm characteristics such as the percentage of motile and morphologically normal sperm are simple and useful qualitative indicators of spermatogenesis, they are not as easy to measure as sperm count.

If sperm count is not the best indicator of male fertility potential, it is an interesting biomarker of testis function and more precisely of spermatogenesis and Sertoli cell number, size, and activity. There is a significant relationship between the number of Sertoli cells and the number of spermatozoa produced. Therefore, any factor that alters Sertoli cell multiplication and differentiation during testis development or irreversibly damages Sertoli cells postpuberty will reduce the number of spermatozoa produced by the testis.

5.1.2.2 Are there temporal and/or geographical trends in human sperm quality and testis function? The issue of whether particular regions show temporal declines in sperm count and testis function has been discussed over a long time, not just in relation to more recent concerns about EDCs. It has been hypothesized that there is a "global" or worldwide decline, but this is not supported by the information available to date.

A possible decline in human sperm quality was first suggested by Nelson and Bunge (1974), who reported a mean sperm concentration of 48×10^6/ml in 390 fertile men requesting a

vasectomy in Iowa City, which was lower than the then expected normal values. Rehan et al. (1975) reported that the mean sperm count was 79×10^6/ml among 1,300 fertile men requesting vasectomy in NYC. These results together with the data of a third American study (Zukerman et al., 1977) were reviewed by MacLeod and Wang (1979), who concluded, based on the data from infertile men attending their own laboratory over a 30-year period, that there were no convincing arguments in favor of a temporal decline in human sperm count. However, based on a meta-analysis of 17 articles published from 1934 to 1979 reporting the mean sperm counts of unselected men of proven fertility, James (1980) concluded that there was "good reason for supposing that there has been a decline in *reported* counts."

The question was highlighted again in a 1992 meta-analysis, based on 61 articles, which concluded that the mean sperm concentration of healthy men had declined from 113×10^6/ml to 66×10^6/ml within 50 years between 1938 and 1991 (Carlsen et al., 1992). The principal criticisms of the above meta-analysis were that the men included in several studies could have been very heterogeneous in terms of their geographic location, season of study, fertility, age, and socioeconomic conditions, so they could hardly be compared. Additionally, the sample sizes in many studies were very small and thus did not permit useful comparisons to be made (Farrow, 1994). It was also suggested that the results could be influenced by confounding factors or by differences in the methods used to analyze semen, as well as the statistical methodology used for the meta-analysis.

Reanalyzing the data of the studies included in the meta-analysis of Carlsen and coworkers (1992), Becker and Berhane (1997) found that the decline in sperm count was significant in the USA alone, the only country included in the meta-analysis with data available over 50 years. In a more extensive reanalysis of the data of Carlsen and colleagues (1992), Swan et al. (1997) confirmed a mean significant decline in sperm concentration of 1.5% per year in the USA between 1938 and 1988 and found a mean significant decline of 3.5% per year in Europe between 1971 and 1990. A new analysis of an expanded set of studies by Swan and colleagues (2000) confirms the decline in semen quality and suggests that the trends in semen quality cannot be attributed solely to confounding factors.

The above reports and commentaries have stimulated various laboratories to analyze their own data. Several publications have reported the results of longitudinal retrospective analyses of semen characteristics of more or less homogeneous groups of men recruited in a single center for a long period of time (Table 5.1). The studies noted in Table 5.1 should not be given equal weight, because they vary considerably in experimental design and sample size. It should also be noted that the time spans covered are not as wide and do not go as far back as does the meta-analysis of Carlsen et al. (1992), the majority commencing in the 1970s or 1980s. The

Table 5.1 - Summary of Longitudinal Retrospective Studies of the Sperm Count Made in a Single Center

		Period	Number	Main\of the Men	Concentration	of Sperm
1	Paris (France)	1973–92	1,351	Unselected potential donors for AI, fathers	↓	↓
2	Ghent (Belgium)	1977–95	416	Unselected potential donors for AI, unknown	↓	=
3	Turku (Finland)	1967–94	5,481	Infertile couples	=	=
4	Edinburgh (UK)	1984–95	577	Research donors, fathers and unknown		↓
5	Toulouse (France)	1977–92	302	Unselected potential donors for AI, fathers	=	=
6	Minnesota (USA)	1970–94	662	Prevasectomy, fathers and unknown	↑	NA
6	New York (USA)	1972–94	400	Prevasectomy, fathers and unknown	↑	NA
6	LA (USA)	1978–94	221	Prevasectomy, unknown	=	NA
7	Seattle (USA)	1972–93	510	Research donors	↑	↑
8	Athens* (Greece)	1977–93	2,385	Infertile couples	↓	NA
9	France**	1989–94	7,714	Partners of women with tubal disease	↓	NA
10	Pisa (Italy)	1970–90	4,518	Infertile couples	↓	NA
11	Sydney (Australia)	1980–95	509	Unselected potential donors for AI, fathers	=	NA
12	Odense (Denmark)	1990–96	1,055	Partners of women with tubal disease	=	NA
13	Münster (Germany)	1977–93	187	Research donors	=	=
14	Jerusalem (Israel)	1980–95	188	Unselected potential donors for AI, unknown	=	NA
15	Southern Sweden	1985–95	718	Infertile	↑	=
16	Canada	1984–96	48,968	Infertile couples	=	NA
17	Copenhagen (Denmark)	1977–95	1,927	Unselected potential donors for AI, unknown	↑	↑
18	Barcelona (Spain)	1960–96	22,759	Infertile	=	NA
19	Slovenia	1983–96	2,343	Partners of women with tubal disease	=	=
20	Magdeburg (Germany)	1974–94	5,149	Infertile couples	↓	NA
21	Hamburg (Germany)	1956–80	36,000	Infertile couples	↓	↓
22	Berlin and Leipzig (Germany)	1985–96	3,821	Infertile couples	↓	↓

*Results from three different laboratories in Athens. **Results from 77 centers. Abbreviations: AI, artificial insemination; unknown, unknown fertility; ↓, significant decline; ↑, significant increase; =, no significant change; NA, not available. References: **1**, Auger et al., 1995; **2**, Van Waeleghem et al., 1996; **3**, Vierula et al., 1996; **4**, Irvine et al., 1996; **5**, Bujan et al., 1996; **6**, Fisch et al., 1996; **7**, Paulsen et al., 1996; **8**, Adamopoulos et al., 1996; **9**, De Mouzon et al., 1996; **10**, Menchini Fabris et al., 1996; **11**, Handelsman, 1997; **12**, Rasmussen et al., 1997; **13**, Lemcke et al., 1997; **14**, Benshushan et al., 1997; **15**, Berling and Wolner-Hanssen, 1997; **16**, YoungLai et al., 1998; **17**, Gyllenborg et al., 1999; **18**, Andolz et al., 1999; **19**, Zorn et al., 1999; **20**, Glöckner et al., 1998; **21**, Licht, 1998; **22**, Thierfelder et al., 1999.

origin of the samples is also an important consideration. For example, men requesting vasectomy are not comparable to men not requesting such surgery. Similarly, studies including men from infertile couples should be viewed with caution because criteria for infertility have changed over time, the proportion of couples seeking assistance is now much greater, and treatment options have changed radically in recent years.

Nine studies included infertile men (Vierula et al., 1996; Adamopoulos et al., 1996; Menchini Fabris et al., 1996; Berling and Wolner-Hanssen, 1997; YoungLai et al., 1998; Glöckner et al., 1998; Licht, 1998; Thierfelder et al., 1999; Andolz et al., 1999). All but one included more than 1,000 men (Table 5.1). Declines in sperm count were found in Athens, Pisa, France, and Germany, an increase was found in southern Sweden, no change was observed in Turku and Barcelona, and no change, an increase, or a decrease was found at different centers in Canada. It is impossible to draw any conclusions from these studies because the availability of infertility services and the behavior of couples wanting to become parents have changed tremendously during the last 30 years in the European countries where most of these studies were done. For example, a laboratory becoming more specialized in male infertility would increase the proportion of severely infertile men recruited, resulting in a decrease in mean sperm count.

Six studies included unselected potential donors for artificial insemination. In three of them (Paris, Toulouse, and Sydney), all the men were fathers (Auger et al., 1995; Bujan et al., 1996; Handelsman, 1997); in the other three studies (Ghent, Copenhagen, and Jerusalem) they were mainly young students of unproven fertility (Van Waeleghem et al., 1996; Gyllenborg et al., 1999; Benshushan et al., 1997). All these studies were apparently done with standardized methodologies, considering only the first ejaculate and using suitable statistical methods. However, in four of them (Ghent, Toulouse, Sydney, and Jerusalem), the sample size was small; the mean number of men included was less than 35 per year. In two cities (Paris and Ghent), a significant decline in sperm count was found; in three others, there was no change (Toulouse, Sydney, and Jerusalem), and in Copenhagen a significant increase in mean sperm concentration and total sperm count was found. A decrease in the total sperm count was observed only in Paris. Despite rigorous methodologies, sample size limitations preclude firm conclusions and only suggest that there may be geographical differences in semen quality.

Three studies analyzed the temporal trend in semen characteristics of donors recruited for research purposes. A decline in mean and total sperm count was found in Edinburgh (Irvine et al., 1996), but an increase in the same characteristics was found in Seattle (Paulsen et al., 1996). The latter study included men with only one ejaculate and men with multiple ejaculates where mean semen characteristics were calculated. In a third study in Münster, no change was observed (Lemcke et al., 1997).

One study analyzed cryopreserved semen taken from men prior to vasectomy in three U.S. cities (Fisch et al., 1996). An increase in sperm concentration was found in Minnesota and NYC; no change was found in LA. In addition, very different mean sperm counts were found (131.5×10^6/ml in NYC, 100.8×10^6/ml in Minnesota, and 72.7×10^6 in LA), but these differences were not analyzed statistically (Fisch et al., 1996). Moreover, in these three cases the data were collected from very small samples (<20 per year in NYC and LA) for which the fertility status was heterogeneous and often unknown. Additionally, the methodology used to count the sperm differed over the time frame of the study (17–25 years), and the reasons for requesting vasectomy may also have changed over time. Given these limitations, it is not possible to conclude whether there are any real differences between the sperm counts found in these three cities.

Most of the retrospective studies report results from a single center. It is of interest to compare the results obtained from different centers located in the same geographic area, that include healthy men studied at the same time period or at different times. Data from three studies in NYC offer the opportunity to compare sperm concentrations over time (MacLeod and Gold, 1951; Rehan et al., 1975; Sobrero and Rehan, 1975; Fisch et al., 1996) (Table 5.2). MacLeod and Gold (1951), using sperm collected between 1945 and 1951 from partners of pregnant women, reported a mean arithmetic sperm concentration of 107×10^6/ml, with 7% of the men having sperm concentrations below 20×10^6/ml. Rehan et al. (1975) in a study of men who had fathered at least two children and were requesting a vasectomy between 1969 and 1974 found a decline in mean arithmetic sperm concentration to 79×10^6/ml, with 5% of the men having sperm concentrations below 20×10^6/ml. Fisch et al. (1996), in a study of prevasectomy patients between 1972 and 1994, found a mean arithmetic sperm concentration of 131.5×10^6/ml. Several methodological differences may account for the discrepant results. First, the earlier two studies analyzed only the first ejaculate of the men, whereas Fisch et al. averaged multiple ejaculates for an unknown proportion of the men they studied. Thus, a bias toward inclusion of men with higher sperm concentrations may have been introduced (Auger and Jouannet, 1997). Second, because Rehan et al. did not control for fertility potential at the time of semen sampling, they performed a subanalysis of 100 men whose partner was currently pregnant and found similar results. Finally, no study accounted for differences in sperm counting methodology.

The studies analyzed by James (1980), Carlsen et al. (1992), and Swan et al. (2000) and other more recent studies illustrate the very large differences in mean sperm concentration reported in populations of healthy and/or fertile men in various parts of the world (Jouannet et al., 2001). It varies from 131.5×10^6/ml in NYC (Fisch et al., 1996) to 48.5×10^6/ml in Iowa City (Nelson and Bunge, 1974), 52.7×10^6/ml in Thailand (Aribarg et al., 1986), and 54.7×10^6/ml in Nigeria (Osegbe et al., 1986). These

Table 5.2 - Sperm Concentration of Healthy Men in NYC (USA)

Reference	Period under Study	Number of Men	Main Characteristics	Mean Sperm Concentration ($\times 10^6$/ml)	
				Arithmetic	Geometric
MacLeod and Gold, 1951	1945–51	1,000	Partners of pregnant women	107	90
Rehan et al., 1975	1969–74	1,300	Prevasectomy, fathers	79	65
Sobrero and Rehan, 1975	1969–74	100	Partners of pregnant women	82	68
Fisch et al., 1996	1972–94	400	Prevasectomy, fathers and unknown fertility	131.5	NA

NA, not available.

differences suggest a possible geographical diversity in human sperm production. Understanding this diversity could help to clarify the influence of environmental or occupational factors on testis function.

In a study analyzing the semen characteristics of men who attended 11 university centers in Canada from 1984 to 1996, YoungLai et al. (1998) found a significant difference in the mean sperm concentration between centers ranging from 48.6 to 104.5 $\times$ 10^6/ml. The mean sperm count increased over the time frame examined in some centers, decreased in others, while still others remained unchanged. Hence, this study not only supports the hypothesis that there are regional differences but also indicates that factors influencing semen quality may be exerting widely divergent effects in different regions.

In 4,710 unselected fertile potential semen donors in eight different centers for the study and preservation of human eggs and sperm (CECOS) in France, it has been shown that the mean sperm concentration (n) and the total sperm count (N) were significantly different (Auger and Jouannet, 1997). The highest values were found at Lille in northern France (n = 103 $\times$ 10^6/ml; N = 398 $\times$ 10^6), and the lowest values at Toulouse in southern France (n = 86 $\times$ 10^6/ml; N = 259 $\times$ 10^6). All the centers were in university hospitals and employed similar criteria for donor recruitment and semen analysis methods. After adjustment for age and period of abstinence before semen collection, the differences between the eight centers remained statistically significant.

The first study specially designed to measure geographical differences in semen quality has recently been completed in Europe, comparing semen characteristics of homogeneous populations of partners of pregnant women (Jørgensen et al., 2001). This cross-sectional study showed significant differences in mean sperm concentration between fertile men recruited from Copenhagen (77 $\pm$ 66 $\times$ 10^6/ml), Edinburgh (92 $\pm$ 63 $\times$ 10^6/ml), Paris (94 $\pm$ 72 $\times$ 10^6/ml), and Turku (105 $\pm$ 73 $\times$ 10^6/ml). The same differences were observed in mean total sperm count from 276 $\pm$ 240 $\times$ 10^6 in Copenhagen to 412 $\pm$ 312 $\times$ 10^6 in Turku. Interestingly, the differences were still significant after adjustment for age, abstinence delay, and season of study. Significant differences have also been reported between various districts of the same city such as London (Ginsburg et al., 1994) or of the same region such as the Paris area (Auger and Jouannet, 1997).

Viewed as a whole, several of the published reports support the hypothesis that there are time-related decreases in semen quality at least within some regions, as reflected in sperm concentration and, where measured, sperm motility and morphology but do no support the hypothesis that the decline is worldwide. Significant differences between regions in semen quality have been demonstrated. However, the biological significance of these observations and their potential causes remain to be determined. Any relationship with endocrine disruption, although plausible, remains entirely speculative at this time.

5.1.2.3 Factors that may influence human sperm quality and bias interpretation of studies on trends. As for most semen characteristics, sperm concentration varies greatly between and within individuals. There is a 10-fold difference between the 10th percentile and the 90th percentile of the distribution in fertile men (Jouannet et al., 1981). The causes and the consequences of this variability on fertility have been well discussed in recent publications (Mees et al., 1997; Auger and Jouannet, 1997; Jegou et al., 1999; Jouannet et al., 2001; Weber et al., 2002). Factors that may influence sperm count and that may explain part of the variability can be classified into three categories: those linked to the characteristics of the men included in the studies, those depending on the methodologies used to analyze the semen or to analyze the data, and those depending on external factors, influencing testis function. Factors relating to the first two categories are listed in Table 5.3. Either information on these factors was not available in most of the reported retrospective studies, or they were poorly considered during the data analysis. The definitions of the populations were particularly vague.

Genetic factors cannot explain the rapid temporal trends that have been described but could explain geographical variations. For example, it is known that Y deletions may be responsible for major spermatogenesis defects (Ma et al., 1993; Reijo et al., 1995). Similarly, differences in gene composition or activity may explain, at least in part, the variability of sperm production within healthy men. Polymorphisms in genes of the Y chromosome have been shown to influence sperm concentration in healthy, fertile Japanese men classified according to their Y haplotypes (Kuroki et al., 1999), and the proportions of men with particular haplotypes varies with ethnic origin (Shinka et al., 1999). It has been demonstrated that fertile brothers of infertile men have lower sperm counts and quality than other fertile men (Czyglik et al., 1986). This observation, which was the first to identify a clear familial component to normal human spermatogenesis, has since been confirmed by Auger et al. (1995). In a study of 17 twin pairs (11 monozygotics and 6 dizygotics), sperm concentration, total number of sperm, and testis volume showed strong familial congruence (Handelsman, 1997). Although these sibling studies do not distinguish between genetic or shared environmental influence, they suggest that a familial factor should be taken into account as a source of variability in the comparison of sperm quality. The very low mean sperm count of fertile men from Africa or South East Asia (Osegbe et al., 1986; Aribarg et al., 1986; Chia et al., 1998) also suggests that an ethnic factor could influence spermatogenesis. The available data, however, do not permit determination of the relative contribution of the respective influences of ethnic, environmental, or other factors, such as sexually transmitted disease incidence, to the reported differences.

In order to draw valid conclusions from comparison of semen values between various studies or within a single study, the sample size analyzed and the role of interindividual variability in sperm production should be delineated. Very large groups of men are required to measure temporal or geographical variations (Berman et al., 1996). In the meta-analysis published by Carlsen et al. (1992), 12 studies included fewer than 20 men and 29 included fewer than 50 men. In 8 of the 10 longitudinal retrospective studies on healthy men published after 1994, the mean number of men included per year was less than 50 (Table 5.1). In such a situation, small changes

Table 5.3 - Factors That May Influence Semen Characteristics Apart from Environmental Chemicals

Population Characteristics	Methodology
Origin of the men	Mode of semen collection
Occupation	Semen analysis methods
Age	Number of ejaculate
Previous medications and diseases	Sexual abstinence delay
Dietary, clothing, smoking habits	Intra- and intertechnician variability
Stress	Season
Fertility	Statistical methods
Sexual activity	
Excessive heat exposure	

in the study or sample characteristics may have an important influence on the temporal trend (Auger and Jouannet, 1997). It is also important to limit the influence of thd variability between technicians and/or laboratory on semen analysis results. A quality assessment made by the four trained European groups who participated in the cross-sectional study cited earlier (Jørgensen et al., 2001) calculated that if the number of samples is 100, a difference of 26% in the geometric medians of sperm concentration will result in a statistically significant difference at the 5% level. If the number of samples is 500, a difference of 18% will be statistically significant (Jørgensen et al., 1997). The size of the populations, their homogeneity, and/or standardization of several factors are important in order to compare data between locations or from one time to another (Table 5.3).

None of the published studies is representative of the general population (Handelsman, 1997). Self-referred volunteers are often better educated people or men with doubts about their own fertility and consequently eager to participate to get information on their own health status. Although it is impossible to make a valid evaluation in the general population, it would be valuable to determine if the same trends exist in various well-defined categories of men, such as young military conscripts, fertile partners of pregnant women, unselected sperm donors, men cryopreserving sperm before a vasectomy, or unselected infertile men.

Although the methodological parameters listed in Table 5.3 are not always taken into account in most studies, they need to be carefully addressed. For example, because of the large intraindividual variability in sperm concentration, comparisons based on the means of several timed ejaculates from each subject would be more appropriate. This, however, may select the men with the highest sperm counts (Auger et al., 1995).

Control for confounding factors, such as age, sexual abstinence delay, or seasons, could be introduced in the statistical analysis to enable better comparisons of data from various centers or times. This has been done in several studies presented in Table 5.1, although the details given are rarely sufficient to permit calculation of adjusted values, which would allow better comparisons of the data. In the study comparing the semen quality in eight different centers in France (Auger and Jouannet, 1997), the adjustment of data to account for men's age and duration of sexual abstinence before semen collection clearly lowered the range of variations of mean and median characteristics between centers. These factors should be included in multiple regression models to compare data of various populations and to measure the effect of external factors.

5.1.2.4 Evidence from hormone and chemical exposures in humans. The Sharpe and Skakkebaek (1993) hypothesis would predict that exposure to hormonally active drugs during gestation should have an effect on later sperm quality, provided free hormone reached the embryo and fetus. The consequences of *in utero* exposure to DES on male reproductive function has been reviewed by Swan (2000). Although sons of DES mothers have an increased incidence of reproductive tract abnormalities, the results for semen quality are conflicting because some studies found reductions in semen quality and others did not. Fertility does not appear to be affected (Wilcox et al., 1995). The fertility of offspring, born between 1958 and 1963, with *in utero* exposure to pharmaceutical estrogens and progestins other than DES, was examined in a retrospective study of 1,888 men and women and 2,044 age-matched controls in Finland (Hemminki et al., 1999). No significant impact of *in utero* exposure to estrogens and progestins on the fertility of offspring could be demonstrated in this study, except that fewer exposed than unexposed men had their first live-born child within one year after getting married. The semen quality of the men was not analyzed.

In an analysis of the semen of men attending an infertility clinic in Calgary, Canada, in 1990–1991, a lower sperm concentration and motility and an increased rate of abnormal sperm (tapered head) were found among 55 agricultural workers. However, the difference in sperm concentration was not statistically significant when compared with the influence of other occupational factors; a more significant dose–response relationship was found between the level of perceived job stress and various abnormalities of sperm morphology and motility (Bigelow et al., 1998). Moreover, this is a difficult study to interpret since attendees at infertility clinics all have the potential for abnormal sperm. In another study, although a significant decline in median sperm concentration was found in Danish agricultural workers after the spraying pesticide season compared with before the season, an equal decline was found in the control group where men were not spraying pesticides (Larsen et al., 1999).

Two studies have been done to analyze the relation between semen characteristics and the concentration of environmental chemicals in seminal plasma. Dougherty et al. (1980) found a negative correlation between the sperm count and PCBs and other organochlorine compounds in the semen of 132 healthy students. A study in 170 men with variable fertility status found that the concentration of PCB congeners was inversely correlated with sperm motility in samples with a sperm concentration of $<20 \times 10^6$/ml (Bush et al., 1986).

5.1.2.5 Conclusions on sperm quality and testis function. Although an extensive review of the published data suggests that there could be temporal and geographical variations in human sperm production, it is not possible to conclude that the phenomenon is real and, if so, to what extent reductions in sperm count may affect fertility. The data should be interpreted with caution. To date, most of the published studies have been retrospective and report data from men recruited for other purposes (donation for infertile couples or research, prevasectomy cryopreservation, infertility diagnosis, etc.) rather than for the analysis of temporal or geographical variations. Recruitment could be very different in the various centers. Additionally, many biases, depending on the methods of analysis employed or the characteristics of the men, could influence the results. These, however, were not taken into account in most studies. Cross-sectional studies of well-defined populations are already underway in several countries but tend to have poor participation rates (Jørgensen et al., 2001).

As discussed above, biologic plausibility and experimental evidence support the hypothesis that chemical agents acting as EDCs could induce testis dysfunction. Moreover, the increasing trends seen in the incidence of testicular cancer (section 5.4.4) and possibly male reproductive tract abnormalities (section 5.1.6) (outcomes that share similar developmental origins and influences) increase the biological plausibility of an EDC influence on a constellation of effects, including sperm quality. However, the lack of any clear effect of the pharmaceutical estrogen DES on human testis function and the lack of any demonstration to date of an endocrine-disrupting mechanism for other chemical exposures indicate the need for more studies before firm conclusions can be drawn. Outcomes in relation to exposure to EDCs need to be analyzed at two different times – exposure before birth or perinatally, when EDCs could alter the testis and genital tract development, and exposure after puberty, when a toxic effect could impair spermatogenesis.

5.1.3 Fecundity and Fertility

5.1.3.1 Methodologies showing temporal and geographical trends. Fecundity, fertility, and "sperm quality" are distinct parameters that are not equivalent and are frequently confused. Sperm count and sperm quality do not necessarily predict whether conception will take place for a given couple. A "fertile couple" has conceived at least one child. Fecundity is the ability of a couple to conceive a child and is often evaluated by the time necessary to achieve pregnancy. "Time to pregnancy" is a useful epidemiological tool to measure the fecundity of a population. It does not require categorization of subjects into fertile and infertile groups—a method with significant limitations. The model has been validated as a consistently effective tool for measuring the impact of exogenous agents that affect reproduction. For example, studies have shown that there is a clear difference in the time to pregnancy among nonsmokers and smokers (e.g., van der Pal-de-Bruin et al., 1997).

Because of the complexity of human reproduction, it is often difficult to determine whether or not there is an actual increase in age-specific infertility rates. Published studies on temporal or geographical trends in human fecundity are sparse. However, the time to pregnancy has been observed to be shorter among couples in Finland than in the United Kingdom (Joffe, 1996), a finding that fits with the view of most published studies that sperm counts are higher in Finland (Suominen and Vierula, 1993; Vierula et al., 1996; Jensen et al., 2001). By comparison, although semen quality has been reported to have declined in the United Kingdom (Irvine et al., 1996), a retrospective study of the British population 16–59 years old on time to pregnancy for all births conceived after unprotected intercourse that began during 1961–1993 has revealed an increase in fertility rather than a decline (Joffe, 2000). Because parity and body mass index have been used as indicators of maternal estrogen levels, and being the first child and being the son of a moderately obese mother are known risk factors for cryptorchidism and testicular cancer, it was reasoned that these factors may also be associated with decreased fecundability (Joffe and Barnes, 2000). In a cohort study using a representative sample of the Brhtish population born in 1958 that have been followed since birth, the effect of the age of the parents of subjects at the time of their birth, as well as numerous other characteristics (smoking, body mass index, height, parity of mothers, and social class), on time to pregnancy was evaluated. A total of 1,714 men and 2,587 women provided data on time to pregnancy. All of the ORs were in the vicinity of 0.9–1.1. Even *in utero* exposure to cigarette smoke was not associated with any effect on female time to pregnancy as an adult, in contrast to previously published reports. These data therefore suggest that the heterogeneity in fertility is not related to any of the factors examined in this study. Thus, no clear picture emerges with respect to time trends and human fertility.

Differences in time to pregnancy have been found in a prospective study involving seven well-defined geographical areas in Europe (Juul et al., 1999). The highest fecundity was observed in southern Italy and northern Sweden; the lowest fecundity was in east Germany. The differences in time to pregnancy remained significant after adjustment for regional differences in body mass, smoking, frequency of intercourse, and sexually transmitted disease. The longest time to pregnancy was observed in Paris, and the shortest in Rome; however, no sperm data are available from the same European regions to allow association of the observed difference in time to pregnancy with a male factor. A subsequent European study has compared time to pregnancy and sperm quality in fertile couples from Copenhagen (Denmark), Paris (France), Edinburgh (Scotland), and Turku (Finland) (Jensen et al., 2001). After adjustment for confounders, this confirmed a decreased probability of conception in Paris couples compared with couples from the other three cities, where there were no differences in time to pregnancy. The difference was not due to any difference between regions in sperm quality. The authors noted the low participation rates could have resulted in selection bias.

In Sweden, analysis of birth registries has shown that the population of subfertile women (defined as those who did not become pregnant after more than 1 year) has actually decreased from 12.7% in 1983 to 8.3% in 1993 in the general population (Akre et al., 1999a). The decrease, which was independent of maternal age, could not be associated with any trend in semen quality but was probably linked to a decrease of sexually transmitted disease incidence in Sweden.

Another potentially useful approach is to review the total fecundity of a population of people with no predisposition for limitation of family size. For example, there has been a declining age-specific fertility rate in the Hutterite population, a group in which reproductive practices are unlikely to have changed over time (Nonaka et al., 1994; Sato et al., 1994). These retrospective cohort studies revealed a decline in the total number of children born beginning with a cohort 1931–1935 and a continuing decline with subsequent birth cohorts. Although genetic influences cannot be excluded, neither can extraneous factors such as man-made chemicals, and covert birth control practices, although there is no clear link to an endocrine disruptor mechanism.

5.1.3.2 Evidence from chemical exposures in humans. Occupational exposures are often cited as evidence of external impacts on fertility (Schaumburg and Molsted, 1989; Sitarek and Berlinska, 1997; Feinberg and Kelly, 1998). The literature on chemical and other occupational exposures is extensive. Only the studies potentially relevant to EDCs are reviewed here. Published studies relate to the impact of both female and male occupations. In one case–control study, in which 281 women with a diagnosis of infertility were compared with 216 postpartum women, women with a history of working in the agricultural industry had an elevated risk of infertility (Fuortes et al., 1997). Several epidemiological studies have been undertaken to study the fecundity and fertility of farmers exposed to pesticides. A retrospective study of 43 couples in The Netherlands whose respective male partner was a fruit grower included 91 pregnancies from 1978 to 1990 (de Cock et al., 1995). Exposure to pesticides was determined by self-reported data. An adverse effect of pesticide exposure was found, mainly apparent in highly exposed men who tried to conceive during the spraying season. The incidence of couples consulting a physician because of a fertility problem was also much greater in the high-exposure group; however, there is no clear link to an endocrine disruption mechanism. In a follow-up on-going case–control study on occupational exposures and semen quality among couples consulting an infertility clinic, among 899 men who delivered a semen sample, an association between impaired semen parameters and aromatic solvent exposure was observed, but no association was found with pesticide exposure (Tielemans et al., 1999a). However, significantly decreased fertilization rates were observed for couples with male partners exposed to pesticides and enrolled in an *in vitro* fertilization program (Tielemans et al., 1999b). Adjustment for paternal or maternal smoking habits, caffeine use, alcohol consumption, or other occupational exposures had little effect on the observed

association. In a retrospective study of 2,012 farm couples, no strong or consistent pattern of association of exposure to various classes of pesticides with time to pregnancy could be observed (Curtis et al., 1999). Similarly a large study in Denmark and France on exposure to pesticides and a control group of agricultural workers did not demonstrate any effect of pesticide exposure on time to pregnancy (Thonneau et al., 1999). Although no association with endocrine disruption has been demonstrated in any of the foregoing studies, it can be concluded that human fecundity and fertility are reduced in some occupational groups with exposure to man-made chemicals and that there is a need to undertake mechanistic studies to better characterize the association.

In addition to occupationally exposed individuals, at-risk groups may also include those whose social or leisure activities lead to higher exposures than occur in the general public. Changes in fecundability were investigated in the New York State Angler Cohort Study and lifetime exposure to PCBs was estimated from recent consumption of contaminated Great Lakes fish (Buck et al., 1999). Maternal consumption of fish for 3–6 years was associated with reduced fecundability (OR, 0.75; 95% CI, 0.59–0.91). However this effect was no longer significant in those with more than 7 years of fish consumption (OR, 0.75; 95% CI, 0.51–1.07). Maternal consumption of more than one fish meal a month was also associated with reduced fecundability (OR, 0.73; 95% CI, 0.54–0.98), whereas there was no association with paternal fish consumption or with either maternal or paternal estimated lifetime exposure to PCBs. These data suggest that maternal consumption of contaminated fish may reduce fecundability but are insufficient to draw any conclusions about paternal consumption.

Results differing from those above have been reported by others on the relationship between time to pregnancy and the consumption of sport fish containing PCBs and mercury (Courval et al., 1999). In this study, lifetime exposure to PCBs and mercury was estimated by the numbers of fish meals consumed. An increased time to pregnancy was observed with increased paternal consumption (adjusted ORs were 1.4, 1.8, and 2.8 for 1–114, 115–270, and 271–1,127 annual fish meals consumed, respectively). Only in the highest fish consumption group did the 95% CI for the OR exclude 1.0. No relationship was found for women consuming sport-caught fish. These data suggest a weak association only between high fish consumption in men and conception delay.

The studies have obvious weaknesses in that they assume a constant pattern of consumption, that levels of contamination in fish remained constant over time, that the same types of fish were consumed throughout, and that the individuals' susceptibility to adverse effects was constant over the periods under investigation. Nevertheless, these studies indicate that anglers may be an at-risk population for the effects of EDCs from fish.

The possible influence of dietary phytoestrogens should also be considered. It has long been known that grazing on clover-rich pastures high in phytoestrogen precursors causes infertility in sheep (Bennetts et al., 1946). More recently, studies have shown prolongation of the menstrual cycle in healthy premenopausal women given soy protein daily containing 45 mg of isoflavones, attributed to prolongation of the follicular phase due to supression of the normal midcycle surge in FSH and LH (Cassidy et al., 1994, 1995; Lu et al., 1996), although in another study on flaxseed ingestion it was the luteal phase that was reported to be prolonged (Phipps et al., 1993).

Actual chemical exposure data in relation to fertility are limited. One example of what might be possible using new reproductive technology techniques is the isolation of persistent organochlorine chemicals from ovarian follicular fluid of women undergoing *in vitro* fertilization (Jarrell et al., 1993a, 1993b). Isolation of such chemicals at a critical period of oocyte development may provide an important biomarker of exposure and potential outcome.

5.1.3.3 Evidence from animal studies. There are numerous animal study protocols designed to identify the potential hazards of chemical exposure to fertility. One of these, RACB has been used extensively, although it should be noted that RACB was not designed specifically to detect EDCs. The data for 72 chemicals contained in the RACB database were evaluated (Chapin et al., 1997b) to identify which of the definitive measures (sperm counts and necropsy results) were the most predictive of apical concerns (fertility). Longer estrous cycles in mice were correlated with reduced numbers of pups, a relationship that was stronger in F_1 than in F_0 generation animals and not seen in the controls. Fertility was reduced if >15% of the sperm had abnormalities or sperm motility was <37%. These estimates were not improved by including sperm count in the model. The data suggest that epididymal sperm counts, motility, estrous cycle length, and testis and epididymal weights are useful although not complete surrogates for overall reproductive function. Epididymal sperm counts correlate well with fertility over numerous studies such that a reduction of 20% results in reduced fertility. Although these studies point to target organs affected by the test compound, they do not provide evidence that endocrine disruption is involved as the primary mechanism mediating the adverse effect.

5.1.3.4 Conclusions on fecundity and fertility. The foregoing studies demonstrate an association between delayed conception and exposure to high levels of environmental contaminants. However, the relationship between changes in time to pregnancy and endocrine disruption remains speculative, due to the complex array of issues that may alter normal human reproduction and result in a longer time to pregnancy. Moreover, time to pregnancy is dependent on the characteristics of both partners (Weinberg et al., 1994). The time to pregnancy model could, however, be useful in providing corroborative evidence in exploring the significance of geographical differences in sperm quality.

5.1.4 Spontaneous Abortion

5.1.4.1 Methodology and pathobiology. The definition of spontaneous abortion is a pregnancy lost prior to viability, typically defined as 20 weeks from the first day of the last menstrual period, of a fetus weighing less than 500 g. The incidence of spontaneous abortion is estimated to be 50% of all pregnancies, based on the assumption that many pregnancies abort spontaneously with no clinical recognition. The difficulties associated with the measurement of early fetal loss have been discussed elsewhere (Lasley and Overstreet, 1998).

The known causes of spontaneous abortion are primarily chromosomal abnormalities in the first trimester (French and Bierman, 1972). In the second trimester, spontaneous abortions are often attributed to uterine abnormalities. Risk factors for spontaneous abortion include advanced maternal age, increasing parity, increasing paternal age, previous spontaneous abortions, therapeutic agents such as chemotherapy, and radiation and anesthetic agent exposures. Tobacco and ethanol exposure also have an impact, alone and in combination, and substance abuse related to cocaine and other drugs may also be associated with fetotoxic responses (Arbuckle and Sever, 1999).

The effects of environmental influences on spontaneous abortion are difficult to explore. A recent review (Arbuckle et al., 1999) has

illustrated the problems of investigating any associations between pesticide exposures and spontaneous abortion and showed that there is a substantial need to improve methodology. Much of the critique is equally applicable to other chemical exposures. There are data gaps regarding the nature of the exposure of women, confirmation of such exposure by chemical analysis in the blood, sample sizes, selection biases from low participation rates, poor differential recall rates among women with spontaneous abortions and control subjects, case ascertainment rates based on time of pregnancy diagnosis, poor confirmation of the existence of pregnancy, and poor control for previous spontaneous abortion. Furthermore, many of the pesticides have not been studied for their relationships to abortion. In most of the studies to date, no measured exposure concentrations are available to correlate with effects (Arbuckle et al., 1999).

5.1.4.2 Evidence from chemical exposures in humans. Of all pesticide exposures, phenoxyherbicides have been studied most. Ashengrau and Monson (1989, 1990) have shown that paternal exposure to 2,4-D increased the risk of abortion. Although DBCP has been principally related to male infertility, it has also been associated with spontaneous abortion (Potashnik et al., 1984). Wives of workers exposed to organochlorine pesticides have shown an elevated risk of spontaneous abortion and stillbirth (Rupa et al., 1991). Levels of DDT were found to be higher among women with abortions or stillbirths in India (Bercovici et al., 1983; Saxena et al., 1983). There is also evidence that organochlorine and carbamate pesticides cross the placenta and possibly cause fetal death (Arbuckle and Sever, 1999).

Savitz et al. (1997) analyzed the outcome of 3,984 pregnancies in 1,898 couples of farmers in Ontario included in the 1986 Canadian Agriculture Census. The man's exposure to pesticide was based on his experience in a 3-month window of time before conception. An increased spontaneous abortion rate associated with reported use of thiocarbamates, carbaryl, and unclassified pesticides was observed. There was also an association between the use of triazines, particularly atrazine, and 2,4-D and an increased risk of preterm delivery.

Women exposed to HCB as children, who developed severe porphyria cutanea tarda, have been followed for approximately 40 years. HCB residues are still present in many of the survivors. During a review of the reproductive outcomes of these women, an unexpected finding was the association of high serum concentrations of HCB levels with high rates of spontaneous abortion. This finding was evaluated in a population-based methodology to include two control populations. The effect was present when all observations were included, indicating the effect was measurable down to concentrations at detection limits (Jarrell et al., 1998). These observations need to be corroborated in further studies.

A causal link has not been demonstrated in any of the above examples between pesticide exposure and spontaneous abortion, nor has an endocrine disruptor mechanism been established. However, a number of pesticides have been shown to have estrogenic, antiandrogenic, or antiprogestagenic activity (Chapter 3, section 3.12). In the context of spontaneous abortion, it is compounds acting on progesterone, critical for implantation and the maintenance of human pregnancy (Csapo et al., 1976), which are of most interest. Interference with either progesterone production by removing the corpus luteum (Csapo et al., 1976) or, alternatively, inhibiting progesterone function by administration of an antiprogestin such as mifepristone (RU-486; Scheepers et al., 1999), can result in a spontaneous abortion. It is also well known that high doses of estrogen, the so-called "morning-after pill," can be used to prevent implantation following unprotected intercourse. These observations suggest theoretical mechanisms by which abortions might be induced by environmental chemicals, but dose considerations cast some doubt on the probability that low-level environmental chemical exposures would have an effect, unless the chemicals were accumulated in the body.

5.1.4.3 Evidence from animal studies. In this context, a comparison of the animal and human data on HCB is of interest. Foster et al. (1995) demonstrated that *in vivo* administration of HCB monkeys reduces serum progesterone concentration in the luteal phase of the cycle. The mechanism is not understood, although it may involve a reduction in steroid-metabolizing function in the ovary or adrenal. It is possible that HCB could be functioning as an endocrine disruptor by reducing luteal phase serum progesterone and limiting fetal survival. The persistence of this chemical in fat could explain the effect observed in women over many years (see section 5.1.4.2).

The effect of EDCs on preimplantation loss in rodents has been investigated in a decidual cell response model (Cummings, 1993). In this model, pregnant rats are dosed with the test compound on GD 1–8 and, sacrificed on GD 9, and the number of implantation sites was recorded. Ketoconazole, an imidazole antifungal agent that has been shown to alter mammalian steroidogenesis, was shown to decrease the number of implantation sites (Cummings et al., 1997). The decidual cell response was blocked in parallel with ketoconazole-induced suppression of serum progesterone. Because the effects of ketoconazole were absent in long-term ovariectomized and hormone-replaced rats, these data reveal that the effects of ketoconazole on the decidual cell response are via direct effects on ovarian steroidogenesis that specifically results in decreased serum progesterone levels. These data therefore provide a potential mechanism whereby EDCs may play a role in early pregnancy loss.

5.1.4.4 Conclusions on spontaneous abortion. In summary, there are substantial gaps in our knowledge about whether exposure to environmental chemicals has an impact on spontaneous abortion rates. Epidemiological studies with more rigorous methodology are required, but the difficulties of detecting early fetal losses, which may be of more relevance than later clinically recognized spontaneous abortions, should not be underestimated.

5.1.5 The Sex Ratio

5.1.5.1 Temporal trends in the sex ratio. The sex ratio is defined as the number of male births divided by the number of female births. It has been suggested that the sex ratio is a potential sentinel marker for population health analysis (Davis et al., 1998). An important assumption is that the sex ratio normally remains stable over a long period of time, although this intuitive position may be challenged with the availability of data from longer studies of populations.

Declining sex ratios (fewer males) have been recorded for a number of regions including Canada (Allan et al., 1997), the United States (Allan et al., 1997; Scialli et al., 1997), The Netherlands (Pal de Bruin et al., 1997), and Denmark (Møller, 1996). Additional information shows apparent declines in the sex ratio in Sweden, Germany, Norway, and Finland (Møller, 1996, 1998). Reductions have also been noted in Latin American countries (Feitosa and Krieger, 1992, 1993). In contrast, a trend toward an increase in the sex ratio has been reported for Italy, Greece, and The Netherlands (Astolfi and Zonta, 1999).

The magnitude of the decrease in the sex ratio over the 20-year period from 1970 to 1990 has been estimated in North America,

where there were around 8,000 fewer males in Canada and 38,000 fewer males in the United States over this period of time (Allan et al., 1997; Scialli et al., 1997). It should be noted, however, that large population studies are required to address this issue. The Allan et al. (1997) study determined that 4.7 million live births over 20 years would be required to measure such reductions with an alpha value of 0.05 and a beta value of 0.9. It is important that population studies on trends in the sex ratio provide such power considerations.

There may be racial differences in sex ratios within countries. Regression analysis of temporal trends in sex ratio of live births between 1969 and 1995 in the United States (Marcus et al., 1998) revealed a significant decline among whites for the 27 years under study (OR, 0.9935; 95% CI, 0.9919–0.9952). In contrast, the sex ratio among blacks during the same time period revealed a significant increase in the sex ratio (OR, 1.0208; 95% CI, 1.0162–1.0254).

Concerns have also been raised that the effects are not homogeneous in the population throughout the entire country. In the Allan et al. (1997) study, the reductions were expressed most strongly in the eastern part of Canada, with the lowest ratios detected in the Atlantic Provinces and Quebec. Astolfi and Zonta (1999) examined the trends for birth sex ratios in metropolitan compared with nonmetropolitan areas of Italy. In this study, there was a negative trend in live born males in metropolitan areas that was significantly different from the positive trend observed for nonmetropolitan areas. There are also variations in the distribution noted in the United States, with significant declines present in four of nine regions (east-north central, west-north central, south Atlantic and Pacific; Allan et al., 1997).

5.1.5.2 Evidence from chemical exposures in humans. Although data are limited on factors that change the sex ratio, there is clear evidence that external influences are associated with such a change. These factors can be grouped into medical, occupational, and environmental. The reported medical factors shown or suggested to reduce the sex ratio include older age fathers and mothers, *in vitro* fertilization, ovulation induction, non-Hodgkin's lymphoma, hepatitis, and multiple sclerosis (Ruder, 1985; James, 1976, 1980, 1994, 1995a, 1996, 1997).

A theory regarding the control of events at the time of fertilization proposes that the hormonal status of the parents at the time conception is a key consideration (James, 1986). Clinical observations are consistent with this theory. For example, there have been numerous reports that ovulation induction with clomiphene citrate in women results in fewer male births. These have been most recently summarized in a large meta-analysis (Jarrell et al., 1993a, 1993b). The processes involved in ovulation induction clearly interfere with baseline hormonal homeostasis. The changes observed in sex ratio following ovulation induction could be due to endocrine disruption at either pharmacological or toxicological levels, from direct effects of the drug itself and/or the effects created by the changing endocrine environment. Another relevant observation is the report of a higher sex ratio when men were administered testosterone during attempts to conceive (Sas and Ezollosi, 1980). Although the sample size was small, the findings are consistent with the relative estrogen:androgen balance.

A common mechanism underlying decreasing fertility, testicular cancer, and changes in sex ratio has been proposed (Jacobsen et al., 2000). In this study, the hypothesis that there is an association between testicular cancer, lower fertility, and sex ratio was investigated using the total population of Danish men born between 1945 and 1980. Between 1960 and 1993, 3,530 men developed testicular cancer; 1,488,957 men born in the same time period and their biological children (1,250,989) served as the comparison group. Men who developed testicular cancer had significantly lower fertility rates than did the comparison group and a significantly lower sex ratio (0.957 compared with 1.053). The reduction in fertility was more pronounced in men with nonseminoma tumor type, whereas the reduction in sex ratio was independent of histological tumor type. The authors concluded that the data support speculation that these abnormalities are linked by as yet undetermined biological mechanisms.

Although it is difficult to establish cause-and-effect relationships in the general population, evidence collected from studies of occupational and accidental exposures to man-made chemicals indicate that they may be involved, although whether it is via an endocrine mechanism has yet to be demonstrated. Certain occupational exposures have been significantly associated with changes to the sex ratio. Men exposed to the pesticide DBCP experienced profound semen deficiency and severe disruption in fertility (Goldsmith, 1997), with significant reductions in the numbers of males born to women whose partners were exposed to DBCP (Potashnik et al., 1984). The proportion of male births observed for the same men prior to exposure was 0.5. However, the mechanism by which this chemical would have changed the sex ratio is not completely understood, although damage to Y-chromosome-bearing spermatozoa has been proposed (Goldsmith et al., 1984), perhaps by a non-endocrine-mediated chelating mechanism. Occupational exposure to organochlorines has also been suggested to alter the sex ratio. A Netherlands study of offspring born from 1978 to 1990 revealed a shift toward daughters when men had workplace exposure to pesticides (de Cock et al., 1995). Exposure to the pesticide vinclozolin, an antiandrogen, has also been implicated (Zober et al., 1995). Other occupational exposures reported to be associated with a change in the sex ratio have included working in the aluminum industry as "carbon setters," "anode setters," or "carbon changers" (Milham, 1993) and exposure to waste anesthetic gases (Wyatt and Wilson, 1973). Changes have also been reported to result from exposure to inorganic borates, alcohol, lead, and solvents (Dodds and Armson, 1997). Five retrospective studies of heavily polluted Scottish residential areas also produced evidence of internal exposure altering sex ratio (Williams et al., 1992, 1995). The pollutants involved included emissions from Akron smelters, steel foundries and incinerators in Scotland between 1975 and 1983. However, for none of these occupational or environmental exposures, including the pesticides, is there evidence as yet that the effect is endocrine mediated.

Further evidence that environmental exposures may influence sex ratio comes from an epidemiological report of a population highly exposed to TCDD from a chemical plant explosion in Seveso, Italy (Mocarelli et al., 1996, 2000; Signorini et al., 2000). In 1976, an explosion released a large cloud that dispersed many kilograms of TCDD. Between April 1977 and December 1984, corresponding to one half-life of TCDD, there was a reduction in sex ratio among those with the greatest exposures (48 girls and 26 boys). The effect was greatest among parents with the highest serum levels of TCDD. In fact, none of the nine couples with the highest serum TCDD levels bore a single male. In a recent report (Mocarelli et al., 2000), an increased probability of having a female birth was found with increasing TCDD concentrations in the serum of fathers ($p = 0.008$), starting at concentrations below 20 ng/kg body weight. Moreover, fathers that were exposed to TCDD when they were 19 years old or

younger were significantly more likely to father a girl than a boy (sex ratio, 0.38; 95% CI, 0.30–0.47). Although much larger than the initial study by this team, the sample size is still rather small for a study of this type. The study was conducted with 239 men and 296 women, from which 346 girls and 328 boys were produced between 1977 and 1996. Because 1985 the sex ratio in this population has returned to expected levels (Needham et al., 1997). It should be noted, however, that these findings are based on small numbers and have not as yet been corroborated, and that there were no changes in the sex ratio noted in the TCDD-contaminated region of Kazakhstan (Hooper et al., 1998) or in those with high exposure to PCBs and PCDFs from consumption of contaminated cooking oils in Taiwan (Rogan et al., 1999). All these studies suffer from potential sample selection bias, and the results are based on very small sample sizes, as well. The mechanism for any TCDD-induced change in the sex ratio has not been determined, and although there is an assumption from other work on TCDD that endocrine modifications are involved, this has not been confirmed. In a related report, HCB, which like TCDD binds to the AhR although at a lower affinity, may have been associated with a reduction in the sex ratio (Jarrell et al., 2000).

5.1.5.3 Evidence from animal studies. In animals, several findings suggest that external factors may influence the sex ratio via disruption of the endocrine system. Vandenbergh and Huggett (1994) have shown that in mice the intrauterine position of the mother affected the sex ratio of the offspring. These findings correlated with maternal anogenital distance. The anogenital distance was longer in females located in the uterus between two males (2M) than in females located between no males (0M). The proportion of males in first litters born to 2M females was 58%, for 1M females 51% and for 0M females 42%. This pattern continued into the second litter. There were no changes in the number of pups born to mothers from different intrauterine positions.

Second, there is a recognized difference in developmental rates of male and female embryos. Male embryos in almost every species that has been studied advance to the blastocyst stage before females. This may provide a mechanism for intrauterine discrimination at the time of implantation. Other studies have shown there are differential rates of cell growth and division between XX and XY mammalian embryos (Tsunoda et al., 1985), and the differences are associated with the Y chromosome (Burgoyne, 1993). Differential growth rates have been observed in mice (Burgoyne, 1993), rats (Scott and Holson, 1977), and humans (Pederson, 1980). This more rapid growth of male embryos exists at the preimplantation stage (Pergament et al., 1994).

5.1.5.4 Conclusions on sex ratio. The limited evidence from animal studies suggests that there may be an intrauterine endocrine event that is responsible for altering sex ratio. Other observations on differing growth rates of male and female embryos offer a mechanism whereby the actions of estrogens, antiestrogens, and antiandrogens may be having a gender discordant impact. It is possible, for example, that a drug such as clomiphene citrate, which binds to the uterus and acts as an antiestrogen, may have an effect that is toxic to the more rapidly developing male embryo.

Where the biological plausibility is most challenged, however, lies in the area of how environmental agents might be associated with the temporal trends noted above. Endocrine disruptors present in the environment may indeed have subtle, long-term effects on biological tissues. However, this is quite different from the extreme disruption of the hormonal milieu that occurs during pharmacological ovulation induction. A further note of caution is offered in the recent report of Vartianen et al. (1999). Extending the analysis to 250 years in Finland, it was suggested that changes in sex ratio antedated any exposures to environmental chemicals. This would indicate that recent associations with the emergence of certain broad environmental exposures may be misleading because of too brief a period of analysis. Nevertheless, there is limited evidence for the suggestion that changes in the sex ratio may represent a general trend in society as a consequence of exposure to EDCs. There is also a broader hypothesis that the slight reductions in sex ratio represent a re-equilibration in response to rising sex ratios and improvements in medical care during this century (James, 1995b, 1998). The hypothesis that endocrine-active compounds affect the sex ratio will require further investigation of their action on the mechanisms associated with sex determination, implantation, and embryogenesis.

5.1.6 Male Reproductive Tract Abnormalities

5.1.6.1 Development of the male reproductive tract. In mammalian species, the default phenotypic sex is female. That is, for a phenotypic male, a whole series of events must be triggered and coordinated to develop the male reproductive system and associated secondary sexual characteristics. A failure of certain genes to be expressed or hormones to act normally result in a female phenotype. Much of the understanding of the key effects responsible for normal development comes from a variety of genetic and gene knockout/overexpression studies, where the absence or overexpression of a particular gene has been associated with failure of the male reproductive tract to develop. When the gonad develops from the genital ridge in the embryo, it has the potential to follow either the female or male route. Key to becoming a male is the expression of the sex-determining gene (sry); this in turn elicits a cascade of events that enable the fetal gonad to develop into a testis (Berta et al., 1990). Other genes implicated in normal differentiation of the male reproductive system include, but are not limited to, steroidogenic factor-1 (Ikeda, 1996; Parker et al., 1996), DAX-1 (Guo et al., 1995; Parma et al., 1997), and a variety of homeobox genes (Lindsey and Wilkinson, 1996; Pellegrini et al., 1997).

In the fetal gonad, the development of Sertoli cells plays a major role in how the reproductive system develops. The Sertoli cell synthesizes Müllerian inhibiting substance (Behringer, 1995), which initiates the removal of the female structures (the Müllerian ducts) that would have formed the uterus, oviducts, and so forth. Sertoli cells also control normal development of the Leydig cells, the site of androgen (testosterone) synthesis, which then plays an important role in the development of the epididymis, vas deferens, and seminal vesicles. DHT, the metabolite of testosterone produced via the action of the enzyme 5α-reductase, appears crucial for the development of the prostate and external genitalia. In rodents, most of these events take place in late gestation (for the rat GD 12–20), whereas in the human fetus most events are in the first trimester of pregnancy. Table 5.4 illustrates the various time lines for the development of the male reproductive tract in rats and humans.

5.1.6.2 Ability of experimental protocols to detect chemicals affecting male development. In experimental protocols employed until recently to study developmental toxicity, late gestation (e.g., in rats from GD 16 onward) was not normally included as part of the exposure period. Thus, for chemicals that were not persistent in the dam, potential effects on male reproductive development could have been missed in examination of the fetuses just prior to term. Testing guidelines have now been

Table 5.4 - Time Lines for Male Reproductive Development: Comparison of the Rat with Human

Rat (GD/PND)	Human	Event
GD 8–10	4 weeks	Germ cells migrate to medial aspect of mesopnephros, move to parenchyma, along with precursor Sertoli cells and interstitial cells
~12	5 weeks	Process above is complete
~14	6 weeks	Sex cords start to develop: Sertoli cells identifiable
	7 weeks	Müllerian duct begins to degenerate (influence of Müllerian inhibiting substance)
18	8 weeks	Wolffian duct development begins (testosterone); initiation of androgen secretion
	Late 8 weeks	Seminal vesicles bud off Wolffian ducts
	9 weeks	External genitalia start to masculinize; continues to late gestation (DHT)
	10 weeks	Prostate develops from walls of urogenital sinus (DHT)
19	11 weeks	Serum FSH starts to rise
	13 weeks	Serum LH starts to rise
21	40 weeks	Birth
PND 15	12 years	Sertoli cells stop dividing; the first spermatocytes are present
±22	Last trimester	Testis descent

modified or are being modified to extend exposure to cover the developmental period from implantation to just prior to term.

Multigeneration and other research protocols do have the potential to detect effects of endocrine-active chemicals on male reproductive development because the critical period of exposure is covered. It is only recently, however, that additional end points now known to be particularly sensitive to hormonal status have been or are being incorporated into these testing protocols (US EPA, 1998a, 1998b; US FDA, 1999; OECD, 1999a, 1999b). Most breeding protocols give indications of male fertility, litter size, and sex ratio, but newer end points, in particular, measurements of anogenital distance, retention of thoracic areolae/nipples, and preputial separation have now been included as indicators of normal male reproductive development sensitive to the potential effects of exogenous endocrine-active agents. In normal reproduction studies, the sexing of rat pups is accomplished by observing the distance between the sex papilla and the anus. In males, this distance is approximately twice that of females and is a function of the androgenic status of the animals. Treatments can alter this end point, which can be easily measured, normally on PND 1. The anogenital distance is also a reflection of the size of the pup, and pup body weight should be used as a covariate in the analysis of these data. In the normal male rodent pup, the anlagen of the thoracic nipples regresses under the influence of DHT. Examination of pups before the hair begins to grow (usually around PND 14) and counting the number of areolae and nipples again provide an indication of the hormonal status of the animals. Thus, for example the classical antiandrogen flutamide will result in a female phenotype for male pups with the presence of all thoracic nipples in males (Chapter 3, section 3.12.2.1). Preputial separation is an index of puberty in male rodents when the prepuce separates from the glans penis after androgen-induced apoptosis. It is an end point that is relatively insensitive to body weight changes. Significant delays or advances indicate a change in the androgen status of the animals on test.

5.1.6.3 Evidence from animal studies. There is now considerable evidence from laboratory animal studies of the adverse effects of exposure of males to estrogenic and antiandrogenic chemicals during critical periods of development. Effects induced by exposure to E_2 and other estrogenic chemicals during the period of male reproductive tract development include reduced testis and epididymis weight, reduced sperm numbers and motility, increased prostate weight, and delayed puberty. Principal manifestations of developmental exposure to antiandrogens include reduced anogenital distance, hypospadias, retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production.

Examples of effects in experimental male animals exposed to endocrine disruptors during the prenatal/perinatal period are summarized in Table 5.5. A more detailed discussion of chemicals with known endocrine activity that can affect male reproductive tract development in experimental animals is given in Chapter 3 (section 3.12).

5.1.6.4 Hypospadias and cryptorchidism.

5.1.6.4.1 Known risk factors. Known risk factors associated with failure of the testis to descend into the scrotal sac (cryptorchidism) include ethnicity, a family history of cryptorchidism, use of analgesics during pregnancy (Berkowitz and Lapinski, 1996), birth order (Møller and Skakkabaek, 1996), and maternal obesity (Berkowitz et al., 1996). Several of these are also risk factors for hypospadias, a developmental abnormality in which the urethra opens on the underside of the penis or the perineum (Akre et al., 1999b). Evidence of a seasonal effect with peaks for cryptorchidism occurring at different times of the year in various studies has been reported (Källén et al., 1986; Berkowitz et al., 1996), although the significance of this finding has yet to be determined.

It has been suggested that early exposure to EDCs could cause abnormalities of the genital tract as well as reduce sperm production and induce testis cancer (Sharpe and Skakkebaek, 1993). Cryptorchidism is a well-known risk factor for later development of testis cancer, and in Denmark it has recently been found that subfertile men also have a higher risk of testis cancer (Møller and Skakkebaek, 1996). These observations support the hypothesis that there is a developmental link between certain male reproductive health disorders.

5.1.6.4.2 Temporal trends. There are reports of temporal increases in the frequency of developmental abnormalities of the male reproductive tract, such as hypospadias and cryptorchidism. However, it should be noted that statistics for the birth prevalence of these abnormalities vary widely. For example, Toppari et al. (1995) reported a range for hypospadias from 0.37 to 41 per 10,000 infants and for cryptorchidism from 3 to 1,340 per 10,000 infants. These differences are probably attributable to differing diagnostic and reporting criteria and to ethnic/genetic differences. Such large differences can make comparisons between studies unreliable, and it should be noted that much of the temporal trend data discussed below rely on just such comparisons. Minor forms of hypospadias have generally been estimated to account for around three-quarters of cases, and a rising trend may simply reflect a more frequent or earlier diagnosis of minor forms over time or an increasing tendency to report them to birth defect registries (Dolk, 1998). Thus, any trend data on hypospadias should be viewed with considerable caution.

A descriptive epidemiological study, which utilized data from various malformation surveillance systems (Hungary, Sweden, Denmark, Italy, Spain, South America, and Mexico), demonstrated an increased prevalence of hypospadias (Källén et al., 1986). There was considerable variation in the incidence reported for the different systems, with South America and Mexico reporting the lowest rates and Hungary and Sweden the highest for the years 1980–1981. Misdiagnosis was evaluated in this study and found to

Table 5.5 - Examples of Endocrine Effects in Experimental Male Animals Exposed to Endocrine Disruptors during the Prenatal/Perinatal Period*

Chemical	Mode of Action	Species and Exposure Period	Doses and Route	Effects**	Reference
E_2	ER agonist	Rat One generation	0.05–50 ppm in diet (0.003–4.12 mg/kg/d)	At 10 and 50 ppm: reduced weight testes and epi didymides; atrophy testes and epididymides; seminiferous tubule degeneration; reduced sperm numbers, motility; no effect on Sertoli cell number	Biegel et al.,1998; Cook et al., 1998
E_2	ER agonist	Mouse GD 13–19	25–300 µg/mouse subcutaneously	At 25 and 100 µg: increased prostate weight	Welshons et al., 1999
17α-Ethinyl estradiol	ER agonist	Mouse GD 0–17	0.002–200 µg/kg/d orally	At 0.02–2 µg/kg/d: increased prostate weight	Thayer et al., 2001
DES	ER agonist	Mouse GD 11–17	0.02–200 µg/kg/d orally	At 0.02–2 µg/kg/d: increased prostate weight	Vom Saal et al., 1997
Bisphenol A	Weak ER agonist	Mouse GD 11–17	2, 20 µg/kg/d orally	At 2 and 20 µg/kg/d: increased prostate weight	Nagel et al., 1997
Nonylphenol	Weak ER agonist	Rat PND 1–18	0.08–8 mg/kg/d intraperitoneally	At 0.8 mg/kg/d: reduced testis, epididymis, seminal vesicle and prostate weight At 8 mg/kg/d: Reduced anogenital distance, cryptorchidism, poor differentiation of seminiferous tubules, reduced in sperm count, motility and fertility	Lee, 1998; Lee et al., 1999
Methoxychlor	Metabolite is ER, antagonist AR agonist	Mouse GD 11–17	20, 2,000 µg/kg/d orally	At 20 and 2,000: µg/kg/d increased prostate weight	Welshons et al., 1999
Methoxychlor	Metabolite is ER agonist, AR antagonist	Rat One generation	25–200 mg/kg/d	At 25 mg/kg/d: reduced growth At 50 mg/kg/d: reduced caudal sperm count At 100 mg/kg/d: delayed puberty	Gray et al., 1989
Methoxychlor	Metabolite is ER agonist, AR antagonist	Rat GD 14– PND 21 or 42	5–150 mg/kg/d by gavage	At 50 mg/kg/d: reduced growth, delayed puberty, reduced testis ,epididymis, seminal vesicle and prostate weight At 150 mg/kg/d: reduced caudal sperm count, reduced sperm motility	Chapin et al., 1997a
DDT	ER agonist	Mouse GD 11–17	18, 180 µg/kg/d	At 18 and 180 µg/kg/d: small testes, altered aggressive behavior, altered territorial behavior	Vom Saal et al., 1995; Palanza et al., 1999
p,p'-DDE	Weak AR antagonist	Rat GD 14–18	10 or 100 mg/kg/d by gavage	At 100 mg/kg/d: reduced anogenital distance, retained nipples, hypospadias, reduced prostate, glans penis and cauda epididymis wt, prostatic atrophy and prostatitis	Kelce et al., 1995; You et al., 1995; Gray et al.,1999b
Vinclozolin	Metabolites AR antagonists	Rat GD 14– PND 3	3–200 mg/kg/d by gavage	At 3 mg/kg/d: reduced anogenital distance, retained nipples At 50 mg/kg/d: cleft phallus and hypospadias, suprainguinal testes, vaginal pouches, epididymal granulomas, reduced sperm count and fertility At 100 mg/kg/d: small to absent sex accessory glands	Gray et al., 1994, 1999a
Procymidone	AR antagonist	Rat GD 14–PND 3	25–200 mg/kg/d by gavage	At 25 mg/kg/d: reduced anogenital distance At 50 mg/kg/d: retained nipples, hypospadias, vaginal pouch, reduced prostate and seminal vesicle wt, prostatic atrophy and prostatitis	Ostby et al., 1999; Gray et al., 1999b
Linuron	AR antagonist	Rat GD 12–21 GD 14–18	By gavage: 12.5–50 mg/kg/d or 100 mg/kg/d	At 12.5 mg/kg/d: retained nipples, hypoplastic testes At 100 mg/kg/d: reduced anogenital distance, retained nipples, hypospadias, reduced testis, prostate, glans penis, cauda epididymis and epididymides weight, testicular and epididymal atrophy	Gray et al., 1999b; Lambright et al., 2000; McIntyre et al., 2000a, 2000b
Linuron	AR antagonist	Rat One generation	10–40 mg/kg/d by gavage	At 40 mg/kg/d: delayed puberty	Gray et al., 1999b
Dibutyl phthalate	Reduced T in fetal testis	Rat Continuous breeding	0.1–1% in the diet (50–800 mg/kg/d)	At 1% in diet (800 mg/kg/d): Testicular degeneration, epididymis absent/underdeveloped, reduced number of spermatids, reduced mating, fertility	Wine et al., 1997
Dibutyl phthalate	Reduced T in fetal testis	Rat GD 11–21	0.5–2% in the diet (330–660 mg/kg/d)	At 1% in diet (555 mg/kg/d): reduced anogenital distance, undescended testes	Ema et al., 1998
Dibutyl phthalate	Reduced T in fetal testis	Rat GD 3–PND 21 or GD 12–21	100–750 mg/kg/d by gavage	At 250 mg/kg/d: reduced anogenital distance, retained nipples, hypospadias, delayed puberty, epididymis absent/underdeveloped, atrophied seminiferous tubules, reduced spermatogenesis At 500 mg/kg/d: reduced testis weight, absent prostate and seminal vesicles	Mylchreest et al., 1998, 1999; Gray et al., 1999b; Mylchreest et al., 2000
Diethylhexyl phthalate	Reduced T in fetal testis	Rat GD 14–PND 3	750 mg/kg/d by gavage	Reduced anogenital distance, retained nipples, hypospadias, vaginal pouch, reduced testis, prostate, glans penis, cauda epididymis and epididymides weight, testicular and epididymal atrophy	Gray et al., 1999b; Parks et al., 2000
2,3,7,8-TCDD	AhR agonist	Rat GD 15	0.05–1 µg/kg	At 0.05 µg/kg: reduced sperm count At 0.2 µg/kg: delayed puberty At 1 µg/kg: reduced anogenital distance	Gray et al., 1997a, 1997b

*For some of the low-dose effects cited above, other (uncited) authors have been unable to replicate the effects. **Doses in this column indicate lowest dose at which effect seen. Reduced T, reduced synthesis of testosterone.

occur in varying degrees in the different regions. Correction for underascertainment resulted in comparable incidence rates for Denmark and Sweden, two countries with similar social, economic, and reproductive practices as well as pollution levels. If persistent environmental chemicals were contributing to the prevalence of hypospadias, then one would predict that because body burdens increase with age but breast-feeding significantly reduces the maternal body burdens: 1) the incidence of hypospadias should be greater in first-born males, 2) the prevalence will decrease with parity, 3) maternal age at first pregnancy should be associated with an increased prevalence, and 4) zygosity should not contribute meaningfully to the prevalence. In the study of Källén et al. (1986), there was an increased prevalence of hypospadias in first-born males, the prevalence decreased with parity, and maternal age was associated with an increased prevalence. Moreover, although more common in monozygous twins compared to dizygous, the difference was not significant. Two birth defects surveillance systems in the USA also indicated that the prevalence of hypospadias at birth has increased between the 1970s and 1990s (Paulozzi et al., 1997).

However, in England and Wales, the incidence of hypospadias appears to be decreasing following a steady increase between 1965 and 1983 (MRC, 1995). Studies in Finland also have not shown any increase in the incidence of hypospadias. An analysis of the national hospital discharge registry of 1,543 boys born between 1970 and 1986 and surgically treated for hypospadias before 9 years of age from a total of 549,176 male births showed that the prevalence of hypospadias was constant over this time period (Aho et al., 2000). Another study of hypospadias diagnosed at birth in Turku, Finland (Virtanen et al., 2001), found no increase in the rate (0.3%) in a cohort of 5,798 boys born between 1997 and 1999, compared with the rate found by Aho et al. (2000) or compared with the nationwide birth rate of hypospadias during 1993 to 1998. Aho et al. (2000) also discuss the possibility of apparent regional/temporal differences in hypospadias being due to completeness of recording methods.

Analysis of the temporal trends in the prevalence of cryptorchidism also indicates an increase over time. The prevalence of cryptorchidism was investigated by cohort analysis of patient discharge records for the years 1962–1981 for England and Wales (Chilvers et al., 1984) and demonstrated an increase in the prevalence of undescended testis from 1.4% for the 1952 birth cohort to 2.9% for the 1977 birth cohort. This study relied on the rate of orchiopexies carried out each year, and thus it is possible that the entire increase in the described prevalence could be accounted for by a change in the criteria used to select patients for surgery.

In a prospective study (Ansell et al., 1992), 7,441 boys from Oxford were examined for cryptorchidism at birth and then again at 3 months of age during 1984–1988. The cryptorchidism rate at birth was found to have increased by 35.1%, and at 3 months of age by 92.7%, compared with the rates reported for the mid-1950s in 3,612 male infants in London by Scorer (1964). Although direct comparison between these two studies is hampered by different inclusion criteria, because Scorer (1964) included stillbirths and low-birth-weight babies, both of which would increase the incidence relative to the later study, it would seem that the prevalence of cryptorchidism has increased in Great Britain.

A more exhaustive analysis of the trends of both hypospadias and cryptorchidism has recently been reported (Paulozzi, 1999). The birth prevalence rates for hypospadias and cryptorchidism were collected through the International Clearing House for Birth Defects Monitoring System. The rates were systematically and continuously collected in 29 registries from 21 countries recording a total of 4 million births per year. A wide intercountry variation in rates of hypospadias and cryptorchidism around the world was found. A factor of 3 or more could be observed between the highest rates (in USA and Israel for hypospadias, USA and Canada for cryptorchidism) and the lowest rates (Finland, Japan, China, and South America for hypospadias; South America for cryptorchidism). However, differences in methodologies and other factors make comparisons difficult. Although the temporal evolution within various registries suggests an increase in hypospadias rates during the 1970s and 1980s in the USA, Scandinavia, and Japan, no change was observed in Canada. No clear significant increase in cryptorchidism prevalence was observed. For both pathologies a tendency toward a decline of rates was found after 1985.

5.1.6.4.3 Influence of environmental chemicals. In animal experiments, cryptorchidism has been induced with gestational exposure to suspected estrogenic and antiandrogenic chemicals, such as mono-*n*-butyl phthalate in rats (Imajima et al., 1997) and flutamide in pigs (McMahon et al., 1995). Midgestational exposure to TCDD has produced cryptorchidism, reduced germ cell numbers, and epididymal abnormalities in pigs, accompanied by reduced ERα mRNA expression in the gubernaculum and epididymis and increased ERα protein levels in the testis (Barthold et al., 1999). Other experimental examples are presented in Table 5.5 and in Chapter 3 (section 3.12). These all suggest that chemicals with estrogenic or antiandrogenic activity can induce cryptorchidism and hypospadias.

A number of epidemiological studies have suggested that exposure to pesticides may be linked to male reproductive tract abnormalities. An increased rate of orchiopexy has been reported in areas with extensive use of pesticides in intensive farming in Granada, Spain (Garcia-Rodríguez et al., 1996). An increased risk of cryptorchidism and possibly hypospadias has also been reported in Norwegian boys born on farms where pesticides were being used (Kristensen et al., 1997). Increases in urogenital defects have also been noted in children born to those occupationally exposed to pesticides in Colombia (Restrepo et al., 1990) and in Minnesota, USA (Garry et al., 1996). Møller and Weidner et al. (1998) analyzed data from all live male infants discharged from Danish hospitals with a diagnosis of cryptorchidism or hypospadias between 1983 and 1992 and found a significantly increased risk of cryptorchidism but not hypospadias in sons of women working in gardening (OR, 1.7; 95% CI, 1.1–2.4). However, the association accounted for a very small proportion of the total number of cryptorchidism cases (0.3%). No increased risk was found in sons of men working in gardening or farming.

The possible role of the maternal diet in hypospadias has recently been reported (North and Golding, 2000). In this longitudinal population-based study there were 7,928 boys, from which there were 51 hypospadias cases identified by maternal reporting using the records for referral and/or surgery, birth notifications, records of examination by neonatal pediatricians, and post mortem reports. Mothers who were vegetarian in pregnancy had an increased risk of giving birth to a boy with hypospadias compared with omnivores who did not supplement their diet with iron (unadjusted OR, 4.99; 95% CI, 2.10–11.88). Omnivores who supplemented their diet with iron in the first half of pregnancy also had a raised risk (unadjusted OR, 2.07; 95% CI, 1.00–4.32). However, the increased risk was obviated when adjusted ORs were

calculated. Women who reported having influenza during the first trimester also had an increased risk of giving birth to a boy with hypospadias (unadjusted OR, 3.39; 95% CI, 1.50–6.78). It was suggested that vegetarians would have a greater exposure to phytoestrogens than omnivores and this might explain the raised risk in that group. In addition to weaknesses surrounding identification of hypospadias cases, no data are available concerning the amount of phytoestrogens that were consumed by the women or to which the developing fetus was exposed. Other potential sources of exposure were not evaluated in this study.

5.1.6.4.4 Endocrine influences. The development of the male reproductive tract is under sex hormone control (section 5.1.8). Hypospadias and cryptorchidism could therefore be considered as likely markers of endocrine disturbance. Animal studies have demonstrated that exposure to estrogens during development can result in cryptorchidism and hypospadias (Grocock et al., 1988; Vorherr et al., 1979). In humans, the induction of reproductive tract abnormalities (epididymal cysts, cryptorchidism, and other genital abnormalities) in sons of DES-exposed mothers have been well documented (Henderson et al., 1976; Wilcox et al., 1995), but it should be noted that prenatal DES exposure was not associated with hypospadias. However, a meta-analysis of 14 well-selected human studies on the influence of exogenous hormones (oral contraceptives, hormone pregnancy tests, progestagens used in threatened abortion or because of previous miscarriages) has not produced any convincing evidence of an effect of prenatal exposure (Raman-Wilms et al., 1995).

Mutations in the gene coding for the AR are also unlikely candidates as causative factors to explain most cases of either cryptorchidism or hypospadias for the majority of cases, due to the rarity of their occurrence in affected children (Bentvelsen et al., 1995; Sutherland et al., 1996). Lower circulating levels of testosterone have however been demonstrated during gestation weeks 6–14 in boys with cryptorchidism (Garcia-Rodriguez et al., 1996). It is considered plausible that environmental contaminants that increase testosterone turnover, or decrease androgen synthesis, or antagonize androgen action at the receptor level could contribute to an increased prevalence of cryptorchidism.

5.1.6.5 The prostate.

5.1.6.5.1 Effects of developmental estrogenic exposure. The rat prostate undergoes branching morphogenesis after birth (Hayashi et al., 1991) and can be imprinted by endogenous and exogenous hormones during that period (Rajfer and Coffey, 1979). Rajfer and Coffey found that a brief neonatal estrogen exposure permanently imprints prostatic development and is associated with an increased incidence of hyperplasia, dysplasia, and adenocarcinoma with aging. The critical period for persistent effects of estrogen on the human prostate appears to be up to as late as PND 5 and begins before birth (Higgins et al., 1981). Further investigations by Bellido and coworkers (1985) found that males that had been injected with 500 µg of E_2 benzoate on PND 1 exhibited ventral prostate and testicular atrophy, decreased testosterone levels, and a transient increase in prolactin plasma levels on PNDs 15 and 22. Reduced androgen sensitivity and AR levels in adulthood in the ventral and dorsal lobes were also observed following treatment with 25 µg E_2 on PNDs 1, 3, and 5 (Prins, 1987). Therefore, estrogen can block differentiation pathways during development, and this is mediated in part by a block in AR expression in the epithelial and smooth muscle cells.

5.1.6.5.2 Effects of prolactin and E_2 on adult lateral prostate growth. It is well known that estrogen administration in an adult male rat can result in both the regression and growth of the prostate. However, the difference in effect is related to the age and hormonal status of the animal, in addition to the dose of estrogen administered. Estrogen exposure of an intact male results in atrophy of the prostate, which is an indirect effect caused by the suppression of anterior pituitary gonadotropin secretion and subsequent reduction or complete suppression of testosterone secretion (Price and Williams-Ashman, 1961; Mawhinney and Newbauer, 1979). In contrast, estrogen exposure to castrated rats results in an increase in growth, with stromal hypertrophy (Thompson et al., 1979) or epithelial hyperplasia (Salander and Tisell, 1976).

Following E_2 administration in the adult rat, increases in weight, citric acid concentration (Grayhach and Lebowitz, 1967; Walvoord et al., 1976), zinc accumulation (Gunn and Gould, 1956), and uptake of androgen (Tveter and Aakvaag, 1969) were observed only in the lateral lobe. Because prolactin can produce the same effects on the lateral prostate as estrogens in rats (Grayhach and Lebowitz, 1967; Moger and Geschwind, 1972; Negro-Vilar et al., 1977; Holland and Lee, 1980), the effects of estrogen on the stimulation of prostatic growth were believed to be due to increases in the production of pituitary prolactin, because estrogen causes a marked release of prolactin from the pituitary gland (Meites et al., 1972).

Prolactin has been repeatedly shown to stimulate prostatic growth (Grayhach and Lebowitz, 1967; Thomas and Manandhar, 1975; Negro-Villar et al., 1977; Holland and Lee, 1980; Prins and Lee, 1983) and to delay prostatic regression (Kolbusz and Grayhack, 1982). A direct prolactin stimulation of the lateral prostate has been demonstrated using pituitary grafts in castrated males to increase prolactin secretion (Prins, 1987; Schact et al., 1992). However, in these studies the ventral and dorsal lobes were unaffected. This effect was also seen in an *in vitro* study, in which prolactin increased DNA synthesis but only in the lateral prostate (Nevalainen et al., 1991).

The effect of prolactin on the growth of the lateral prostate and the relationship of this effect with androgens was then investigated. Prins (1987) found that prolactin increased endogenous DHT binding to the AR and induced an increase in nuclear uptake of ^{3}H-DHT. It was proposed that there was an increased quantity of androgen-binding sites when serum prolactin is elevated. Prins (1987) also found that the hormonal regulation of ARs differs in the lateral lobe as compared with the ventral and dorsal lobes. The lateral lobe has an androgen-independent component of AR protein expression whereas that of the ventral and dorsal lobes is androgen dependent. Adult exposure to estrogens down-regulates AR expression in the ventral prostate but has no effect on those receptors in the other lobes. Prolactin up-regulates ARs in the lateral prostate specifically and is associated with enhanced growth and secretory activity within that lobe.

5.1.6.5.3 Prostatitis. Prostatitis without a known bacterial origin (nonbacterial prostatitis) is fairly common and poorly defined in humans and is particularly troubling in young and middle-aged men because it can affect fertility (Meares, 1998). A prostatic inflammatory infiltrate is also found in many cases of benign prostatic hyperplasia and prostate cancer (McClinton et al., 1990). Histologically, some strains of aged rats develop a spontaneous prostatitis in their lateral prostates, with an accumulation of neutrophils in the lumina and mononuclear cells in the stroma (Aumuller et al., 1987). In 1988, Robinette showed that E_2 treatment could induce an inflammatory response in the lateral prostate after a 2-week E_2 exposure in the 1-week castrate adult male rat. The existence of hormone-induced inflammation on a long-term basis was associated with subsequent thickening of the

fibromuscular stroma that resembled changes seen in the lateral prostate of the aging rats mentioned above. The presence of this inflammation was correlated with an increase in serum prolactin (Tangbanluekal and Robinette, 1993). The administration of bromocriptine to these E_2-treated males suppressed serum prolactin levels and the inflammatory response seen in the lateral prostates, indicating that prolactin mediated the response (Tangbanluekal and Robinette, 1993). To further characterize this effect, the investigators also found a dose–response relationship between the administration of exogenous prolactin and the severity of the inflammation that was induced.

Estrogen administration to rats postnatally has also been shown to result in ventral prostate inflammation in adulthood (Rajfer and Coffey, 1978; Prins, 1997; Naslund et al., 1988). Other histologic changes that have been observed in adult prostate following neonatal estrogenization include hypotrophic and disorganized epithelium and an increase in stromal elements (Rajfer and Coffey, 1978; Prins, 1997).

Recently, it has been shown that environmental exposures to certain pesticides and environmental compounds during the perinatal, lactational, or early pubertal periods can lead to lateral prostate inflammation or prostatitis in the adult male rat (Stoker et al., 1999a, 1999b, 1999c). These exposures appear to be increasing the secretion of prolactin prior to puberty, which in turn seems to have a relationship with the level of prostatitis observed in the adult male rat.

5.1.6.5.4 Prostate weight and low-dose issues. *In utero* exposures to environmental compounds that have been shown to possess estrogenic activity, such as DES, can induce persistent structural and functional alterations in the developing reproductive tract of male mice (Santti et al., 1998). Recently, there has been much debate over exposure to low doses versus higher toxicological doses of environmental estrogens during the latter half of gestation. Lower doses of bisphenol A and DES *in utero* have been found to increase prostate weight in adulthood, whereas higher doses of the same compounds result in the opposite effect, a decreased prostate weight (Gupta, 2000; vom Saal et al., 1995, 1997; Nagel et al., 1997) (Table 5.5). However, when attempts were made to repeat these studies, none of the adverse effects of low doses of bisphenol A and DES were observed (Cagen et al., 1999; Ashby et al., 1999).

5.1.6.6 Conclusions on male reproductive tract abnormalities. The data on temporal trends in the incidence of hypospadias and cryptorchidism should be interpreted with considerable caution, given the lack of longitudinal studies and the consequent difficulties in comparing data from separate studies in which definition, ascertainment, and registration of these defects may have differed substantially. Data on trends in pathology and possible endocrine-mediated disturbances on the prostate in men, other than cancer, are lacking, although there is now considerable experimental evidence that hormones and other endocrine-active chemicals can affect prostate development.

Studies on environmental chemicals have focused mainly on pesticides. However, most of the human studies have included only small numbers of cases occupationally exposed to pesticides and lacking good exposure data. In some studies, the increase in urogenital defects was part of a general increase in birth defects amongst offspring of pesticide-exposed parents. No conclusions can be drawn on whether there is a causal association with pesticide exposure or whether there is endocrine involvement. However, animal data on several EDCs and on sex hormones and human and animal data on DES clearly demonstrate that hormonal mechanisms can be involved in the etiology of male reproductive tract abnormalities, and the impact of environmental chemicals certainly requires further study.

5.1.7 Endometriosis

5.1.7.1 Pathobiology and the role of estrogen. Endometriosis is an estrogen-dependent disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. It is a common gynecologic disorder as well as a major cause of infertility (Chedid et al., 1995) affecting approximately 14% of women of all reproductive ages (Vercillini et al., 1995). Proliferation of a progenitor stem cell (Meyer, 1897, 1925), or retrograde menstruation of endometrial cells (Sampson, 1927, 1940), or substances shed from the uterine cavity inducing the undifferentiated cells of the peritoneum to undergo endometrial differentiation are thought to lead to the implantation and proliferation of ectopic endometrial cells. At the present time, it is generally appreciated that there is no one single theory that identifies and can explain all aspects of the clinical syndrome. Although retrograde menstruation and bleeding into the peritoneal cavity during menstruation are widely accepted as major contributing factors in the pathogenesis of this disease, it is a common phenomenon even in women without endometriosis (Halme et al., 1984). Hence, factors other than retrograde menstruation are thought to contribute to the development and progression of endometriosis.

There is a clear clinical relationship of endometriosis to endogenous and exogenously administered estrogen and progesterone exhibiting a dose–response effect. The exogenous administration of estrogen has been shown to aggravate the disease. It can be speculated that imbalance of estrogen and progesterone may be involved in the pathogenesis and pathophysiology of this disease. There is evidence that the incidence is reduced through use of oral contraceptives, apparently associated with the inclusion of progesterone and the reduced amount of uterine bleeding. It has been hypothesized that the steady reduction in the estrogen content of the oral contraceptive has resulted in a greater progestin effect that results in a reduced incidence of endometriosis. There is an association of the onset of the disease and progression of the disease with continued menstrual function. The disease can undergo a reduction in symptoms in association with pregnancy but often recurs with the re-establishment of menstrual function. The cessation of menstrual function at menopause is associated with virtual elimination of the disease. Thus, although there is a clear association of severity with estrogen, and it is clear that endometriosis never occurs in its absence, it is generally accepted that the disease is not caused specifically by estrogen but is stimulated by its presence (Guarnaccia and Olive, 1998).

5.1.7.2 Evidence from chemical exposures in humans. Chemical contaminants have been implicated in the pathobiology of endometriosis as a result of a series of clinical-observational and animal studies. In addition, AhR expression has been demonstrated in human endometrium throughout the menstrual cycle (Igarashi et al., 1999). In humans, an association between endometriosis and exposure to PCBs (Gerhard and Runnebaum, 1992) and dioxins (Koninckx, 1999) has been made. A positive association between endometriosis and dioxin exposure was also reported in a single case–control study in which 44 women with endometriosis were compared with 35 age-matched controls with tubal infertility (Mayani et al., 1997). Although significantly more women with endometriosis [8 (18%), vs. 1 woman (3%) of the control group]

tested positive for dioxin in their serum (p = 0.04), there was no relationship between severity of endometriosis and concentration of dioxin. Moreover, although an OR of 7.6 was obtained, the 95% CI included unity (0.87–169.7). In another case–control study, no association between plasma organochlorine concentrations and endometriosis could be found in 86 women with endometriosis compared with 70 controls, matched for the indication of laparoscopy (Lebel et al., 1998). A study of patients presenting for pelvic pain, infertility, and requests for sterilizing tubal fulguration was done to compare the plasma concentrations of organochlorines among those with and without endometriosis. There were no differences found between those with the disease and those without, although there were no power considerations to determine the strength of this negative study (Lebel et al., 1998). The most significantly exposed women in Seveso also underwent an evaluation for endometriosis in a case–control study, which showed there was no association between dioxin levels and the presence or amount of endometriosis in women (Mocarelli et al., 1999). Similarly, a preliminary study of 15 women with endometriosis and 15 controls did not find a statistically significant association with serum levels of PCBs or dioxin (Boyd et al., 1995). These studies are all relatively small, and thus may not have the statistical power to detect differences if they were indeed present. It has been determined that a sample size of 286 subjects with endometriosis and 286 control subjects would be required to detect a twofold increase in the incidence of endometriosis, assuming a 10% prevalence rate, significance level of 0.05 and power level of 90% (Mayani et al., 1997). There are no direct data regarding the impact of phytoestrogens, such as isoflavones or flavanones, on ectopic human endometrium, although it could be anticipated that they might have an effect, given that soy isoflavones inhibit E_2-mediated endometrial proliferation in macaque monkeys. Hence, the human data at present neither confirm nor refute the hypothesis that environmental contaminants play a role in the pathobiology of endometriosis.

5.1.7.3 Evidence from animal studies. The suggested role of dioxins in the pathobiology of endometriosis derives also from a variety of animal experiments. Most notable of the animal studies is a reproduction study in which rhesus monkeys were dosed with TCDD in the diet for 4 years (Rier et al., 2001). Severe endometriosis was discovered in a couple of animals, necessitating necropsy and leading the study team to examine the remaining animals by laparoscopy for the presence of this disease. When the study code was revealed, the prevalence of endometriosis was found to be 33%, 43%, and 71% respectively for monkeys that received 0, 5, and 25 ppt TCDD. These data therefore suggest that TCDD may play a role in the development of endometriosis, although the mechanism was not explored in this study. However, this study has been severely criticized by Golden et al. (1998), and the complexities associated with the use of rhesus monkeys to study this disease have been discussed by McCann and Myers (1970). For example, there is a higher prevalence of endometriosis in rhesus monkeys than in women. In this study, the background rate of endometriosis was identified by historical autopsy records, whereas the incidence in treated animals was determined by laparoscopy. Other factors may also have confounded the results, such as the relative rates of cesarean section, a procedure that increases the rates of endometriosis in monkeys. Although an immune mechanism has been proposed (section 5.4), no data were presented to indicate that these animals could be characterized as having immune suppression. Despite the limitations detailed above, the potential contribution of TCDD to the pathobiology of endometriosis cannot be discounted. Rier et al. (2001) have published further information on the monkeys in their original study, showing that serum levels of TCDD and specific dioxinlike PHAH congeners were increased in TCDD-treated animals with endometriosis 13 years after the TCDD exposure. The animals with high serum levels of the congeners 3,3′,4,4′-tetrachlorbiphenyl and 3,3′,4,4′,5-pentachlorbiphenyl and increased total serum TCDD equivalents (TEQ) had a high prevalence of endometriosis and the severity of the disease correlated with the serum concentration of 3,3′,4,4′-tetrachlorbiphenyl. Other studies have also followed the original study of Rier et al. (2001) in an effort to clarify the role of various chemical contaminants in the etiology of endometriosis.

In a reproductive/developmental toxicology study, no association could be demonstrated between the incidence of endometriosis in rhesus monkeys and treatment with Aroclor 1254 (Arnold et al., 1996). In another study on cynomolgous monkeys (Yang et al., 2000), TCDD treatment (0, 1, 5, and 25 ng TCDD/kg body weight/day) induced a bimodal effect on endometrial implant survival and size. Circulating gonadal steroid levels and menstrual cycle characteristics were unchanged by treatments in this study, and no data were provided on the direct effects of TCDD on the endometrium or immune function.

Although rodents do not spontaneously develop endometriosis, surgical induction of endometriosis in rodents was enhanced by exposure to TCDD (Johnson et al., 1997). The significance of these studies for humans is uncertain. In particular, it should be noted that the effects occurred with relatively high doses of TCDD. Prenatal treatment on GD 8 (3 or 10 µg/kg by gavage), followed by further TCDD treatment (3 or 10 µg/kg by gavage) as adults prior to surgical induction of endometriosis also increased the size of endometriotic lesions in mice but not in rats (Cummings et al., 1999).

In a similar murine model, ovariectomized and controlled for estrogen replacement, administration of 4-chlorodiphenyl, an estrogenic compound, was associated with increased endometriotic cyst growth (Yang et al., 1997). In contrast, Yang and Foster (1997) have shown that the administration of TCDD inhibited the growth of endometriotic cysts in the presence of estrogen and indicated that the experimental model did, however, implicate TCDD as an agent that could influence tissues that are responsive to estrogen. TCDD is a potent AhR ligand. AhR ligand binding affects a number of genes that are potentially important in the pathobiology of endometriosis. In particular, AhR ligand binding regulates EGF, interleukin-1β, TGF-α and TGF-β (Madhukar et al., 1984; Sutter et al., 1991; Gaido et al., 1992). TCDD additionally inhibited the E_2-induced increase in uterine wet weight in the rat (Gallo et al., 1986) and in mice the E_2-induced increase in uterine EGF mRNA levels. Contradictory reports on the effect of TCDD on uterine ER levels; TCDD-induced suppression in rats have been reported (Romkes et al., 1987; Astroff et al., 1990), whereas others found no effect in mice (De Vito et al., 1992). However, decreased expression of ER mRNA in the ovaries and uteri of TCDD-treated mice has recently been demonstrated (Tian et al., 1998).

Molecular mechanisms of TCDD action have been investigated in a nude mouse model bearing implants of human endometrial tissue (Bruner et al., 1997). Human endometrial explants cultured with E_2 were found to secrete stromal- and epithelial-specific matrix metalloproteinases and induced ectopic endometrial lesions when injected into nude mice. However, inclusion of progesterone with E_2 suppressed matrix metalloproteinase secretion *in vitro* and lesion formation *in vivo* (Bruner et al., 1997; Bruner-Tran et al., 1999).

The combination of E_2 and TCDD increased the number and size of endometriotic lesions compared to E_2 alone, whereas TCDD blocked the ability of progesterone to suppress the matrix metalloproteinase secretion and lesion formation *in vivo* (Bruner et al., 1997; Bruner-Tran et al., 1999).

5.1.7.4 Conclusions on endometriosis. In summary, the evidence shows that endogenous and pharmacological doses of estrogens are potential modifiers of endometriosis. However, there are severe constraints on the biological plausibility that TCDD, the main environmental chemical investigated, enhances the disease because it has antiestrogenic effects and exposures would be exceedingly small compared with the high doses used in rodent experiments. One controversial primate study implicating lower levels of TCDD in enhancement of the disease needs further corroboration. Although the study of the survivors of Seveso exposed to high levels of TCDD was negative, this and other human studies were all small, indicating that more powerful analyses are required. Possible mechanisms linking the endocrine and immune systems in this disease also require further study.

5.1.8 Other Adverse Reproductive Outcomes Potentially Linked to EDCs

Environmental chemicals have also been suggested as potential causative factors in the temporal decline in the age of onset of puberty, in polycystic ovarian syndrome, and in shortened lactation.

5.1.8.1 Precocious puberty. The development of secondary sexual characteristics in girls before 8 years of age or in boys before 9 years of age is considered to meet the criteria for a diagnosis of precocious puberty. The current classification of the disorder recognizes 1) central precocious puberty, 2) peripheral precocious puberty, and 3) contrasexual precocious puberty (Bates, 1998). Thus, the hormonal mechanisms are better understood because the abnormal condition is similar to normal physiological developments. Population-based studies showing a reduction in the median age of puberty demonstrate the need for investigations of the possible causes of this trend.

A hypothesized external agent that would induce central precocious puberty would act through the early initiation of the pulsatility of gonadotropin-releasing hormone from the hypothalamus, thereby inducing the cascade of hormonal events that result in pubertal development. However, review of the literature has not established a relationship between central precocious puberty and environmental agents.

In the case of peripheral precocious puberty, the mechanism in girls is through hormonal receptors in peripheral tissues that are responsive to estrogen or estrogenlike compounds. Several reports have indicated there is an association between premature breast development and exposure to DES (Hertz 1979), ethinyl estradiol, and mestranol. This finding has been reported in association with exposure to contaminated meat (Fara et al., 1979; Kimball et al., 1981). DES has been found as a contaminant of baby food containing veal and poultry (Hoffmann, 1982). In Puerto Rico, reports of precocious puberty that have heightened concern that there may be an environmental cause (Mills et al., 1981; Nizzoli et al., 1986; Freni-Titulaer et al., 1986; Hannon et al., 1987; Van Winter et al., 1990). For example, a temporal trend toward premature breast development (premature thelarche) in girls and gynecomastia in boys has been noted in Puerto Rico during the early 1980s. Concerns were expressed that this was due to increased estrogenic compounds from the environment (Mills et al., 1981; Bongiovanni, 1983; Saenz de Rodriguez et al., 1985; Freni-Titulaer et al., 1986). The possible sources considered were foodstuffs and also the presence of waste products from pharmaceuticals because of the high levels of local industrial pharmaceutical production. In a recent report (Colon et al., 2000), serum samples from 41 girls with premature breast development and 35 controls were analyzed for pesticides and phthalate esters. No pesticides or their metabolites could be found in any of the serum samples; however, 28 (68%) of the girls with premature breast development had measurable levels of phthalates [dimethyl, diethly, dibutyl and di-(2-ethylhexyl)], compared with 6 of the 35 (17%) control samples. Although these data suggest that phthalate plasticizers may be associated with precocious breast development in this population, detection of diesters of phthalates in serum would not be expected because they are normaly rapidly metabolized to their respective monoesters before absorption. This and the small sample size suggest that these results should be interpreted with caution. Another study, in which plasma of girls with precocious puberty was screened for pesticides, showed an increase in *p,p′*-DDE in foreign children with precocious puberty immigrating from developing coutries to Belgium, compared with nondetectable levels in Belgian-born girls with idiopathic or organic precocious puberty, suggesting a possible relationship to early exposure to *p,p′*-DDE (Krstevska-Konstantinova et al., 2001).

The effect of *in utero* exposure to PBBs on sexual maturation was evaluated in Michigan girls whose mothers were accidentally exposed through the diet (Blanck et al., 2000). In 1973, the flame retardant FireMaster was inadvertently added to livestock feed instead of the nutritional supplement NutriMaster. Michigan residents subsequently consumed animals and dairy products contaminated with PBBs. Exposure was estimated on the basis of maternal blood samples collected years after the initial exposure. Effects on pubertal end points were assessed by questionnaires sent to mothers of daughters less than 18 years of age and to the daughters themselves. The data reveal that menarche and pubertal hair growth were significantly advanced in girls with high perinatal exposure [unadjusted OR and CI, 0.9, 0.4–1.8 (not breast fed) and 2.1, 0.9–5.3 (breast fed), 0.6, 0.1–2.7 (not breast fed) and 8.4 1.4–50.5 (breast fed), respectively; adjusted OR and CI, 0.8, 0.3–1.9 (not breast fed) and 3.4, 1.2–9.0 (breast fed), and 0.9, 0.2–4.3 (not breast fed) and 19.5, 2.8–138.2 (breast fed), respectively]. There was no association with Tanner stage of breast development. These findings are both interesting and perplexing because menarche and breast development are estrogen dependent whereas pubertal hair growth is dependent. This study suffers from a number of limitations, most notably, the weak exposure assessment that is prone to missclassification error. No firm conclusions can be drawn.

Precocious sexual maturation in rodents has been studied by examining experimental animals for preputial separation in males or premature vaginal opening in females. For example, the age of vaginal opening in Long-Evans rats was advanced by oral exposure to estrogenic chemicals (Laws et al., 2000b). Specifically, advanced age at vaginal opening was found in rats treated orally on PNDs 21–35 with ethynyl estradiol (0.01 mg/kg), methoxychlor (50 mg/kg), 4-*tert*-octyphenol (200 mg/kg), or 4-nonylphenol (50 mg/kg). It does not necessarily follow that exposure to estrogenic compounds will induce precocious puberty as was shown by treatment with bisphenol A, which induces estrogenic responses in the uterotropic assay but at doses up to 400 mg/kg failed to advance vaginal opening (Laws et al., 2000a).

5.1.8.2 PCOS. PCOS, a poorly understood condition, has been described as a self-perpetuating state of chronic anovulation. There is a broad variation of clinical findings ranging from the

classical Stein-Leventhal syndrome to much milder forms of anovulation (Stein and Leventhal, 1935; Ben Shlomo et al., 1995). The syndrome is usually associated with the onset of puberty. The menses may at first be ovulatory, but shortly thereafter oligomenorrhea or amenorrhea ensues. Mild to moderate hirsutism may develop, and obesity is common. The concomitant use of oral contraceptives may delay or reduce the appearance of symptoms. The actual incidence is not established because of the great variation in presentation and the very real possibility of underdiagnosis. However, Polson et al. (1988) have indicated through ultrasound evaluation that polycystic ovaries may be a feature in 22% of young women. The interest in this syndrome in relation to environmental chemicals stems from the animal literature. There is evidence that testosterone exposure has significant effects on the female developing brain, including induction of acyclicity and anovulation (Chapter 3, section 3.3.5). Polycystic ovaries can also be induced in a variety of animal models through estrogen administration (Convery and Brawer, 1991); however, there are no generally acceptable animal models of the pathophysiology of PCOS. Neither are there data on trends in the incidence of PCOS in women.

5.1.8.3 Shortened lactation. Lactation is a critically important process that provides nutrition to the newborn. There are two physiological processes of milk secretion, with let down as well as milk ejection, controlled by a cascade of neurotransmitters from the anterior and posterior pituitary gland. The theoretical problems that endocrine disruption may cause are 1) the transfer of maternal endocrine disruptors to the infant, with subsequent adverse health effects, and 2) induced abnormalities in the production of milk or its release.

Lactation in women can be affected by pharmacological doses of combined estrogen:progestagen oral contraceptives and they are contraindicated until weaning has taken place. A study in a region of northern Mexico, where DDT has been heavily used in agriculture and high human breast milk and fat levels of DDE have been recorded, has shown that the concentration of DDE in the breast milk correlated with duration of lactation. In women with breast milk concentrations of *p,p′*-DDE ≥ 12.5 ppm, the median duration of lactation was 3.0 months, compared with 7.5 months in those with the lowest concentrations of <2.5 ppm (Gladen and Rogan, 1995). Differences remained significant after exclusion of women ceasing lactation for discernible external reasons. In rodents, lactation has been shown to be impaired by exposure to the pesticide atrazine through inhibition of prolactin (Kniewald et al., 1987; Cooper and Kavlock, 1997; Stoker et al., 1999a, 1999b, 1999c). However, no conclusions can be drawn from these sparse human and animal data.

5.1.9 Conclusions and Recommendations on Reproduction

The major limiting factor in drawing any conclusions about human reproductive health effects and putative links to EDCs is the absence of exposure data. Exposure data are very limited, if available at all, and in many studies exposure has only been inferred and not actually measured. Another major problem common to many of the human studies is that sample sizes are often too small to permit detection of an effect, even if one was present. Thus, the currently available human data are inadequate to support a conclusion that human reproductive health has been adversely affected by exposure to EDCs. Similarly, although there is evidence for geographical differences and temporal trends in some aspects of human reproduction, there has been no systematic attempt to look for evidence that the mechanisms behind these changes involve endocrine pathways.

Despite these drawbacks, the biological plausibility of possible damage to human reproduction from exposure to EDCs seems strong when viewed against 1) the background of known influences of endogenous and exogenous hormones on many of the processes involved, and 2) the evidence of adverse reproductive outcomes in from wildlife and laboratory animals exposed to EDCs. The biological plausibility and the striking changes in human reproductive health trends in some areas, for some outcomes, are sufficient to warrant concern and make this area a research priority.

Data on effects of chemicals on female reproductive health are particularly sparse both in the human and experimental literature. Concerns about females derive mainly from biological knowledge about the influence of sex hormones on development and adult reproductive function, rather than from studies on environmental chemicals. Evaluation of possible links to EDCs could be pursued by exploiting identified temporal trends and geographical differences in the sex ratio, whereas the frequency of endometriosis in the general population offers opportunities for human studies requiring fewer numbers than might be required for other end points. Time to pregnancy can be used as an apical tool to broadly examine the possible influence of environmental chemicals on both male and female reproductive function.

With respect to effects on males, several meta-analyses and single retrospective studies suggest that there could be a decline in sperm quality in some regions over time, whereas other studies have not found a decline. Thus, the evidence to date certainly does not support the hypothesis that there is a worldwide decline. Because of the intra- and interindividual variability of human semen characteristics, the heterogeneity of study populations, lack of information defining the study populations, sample size limitations of many studies, and uncertainties of the quality and standardization of most published studies, the possibility of temporal declines in sperm production and fertility in some regions remains open. The geographical variation of sperm count and concentration is a more real phenomenon. The differences observed between large similar populations of healthy men cannot readily be explained by methodological or confounding factors alone. It could be related to environmental factors but also to genetic factors. For the future, prospective studies in well-defined populations and various categories of the population in the same place are needed, in order to determine if changes in testis function are real and extend to the general population. Studies of men exposed to known EDCs are also very sparse. Longitudinal and case–control studies of men exposed to suspected compounds should also be informative, provided other factors that may interfere with male reproductive health are taken into account.

The prevalence of male reproductive tract disorders, such as cryptorchidism and hypospadias, also needs more careful study, particularly because they may be linked to testicular cancer that shows marked temporal increases in many countries. Experimental data in animals suggest that in both the adult and the developing organism, the prostate may be readily influenced by endocrine-active chemicals. To date, there is no human information on prostate changes in relation to early or adult exposures and the difficulties of investigating aspects such as growth and subtle cellular changes in the prostate should not be underestimated.

Because temporal trends in human sperm quality have fueled much of the debate about EDCs and could have a considerable impact within large populations on the proportion of men who are subfertile or infertile, this area warrants high priority for further research.

5.2 Neurobehavior

5.2.1 Introduction

As noted in Chapter 3, the nervous system plays an integrative role along with the endocrine and immune systems in orchestrating important physiologic functions of the body. These integrative functions are critical for normal development, cognitive functions, and behavior. A number of environmental chemicals (including potential EDCs) have been shown to cause neurotoxic effects (IPCS, 1986; NRC, 1992; IPCS, 2001b). A variety of adverse health effects have been observed ranging from motor impairment and memory loss to subtle behavioral changes (Spencer and Schaumburg, 2000). Of particular concern are the potential effects of exposures on the developing nervous system, because both the nature and adversity of the outcome may depend on the time window during which chemical exposure occurs and may result in irreversible neurobehavioral changes later in life (Tilson, 1998).

The complexity of the nervous system as well as its integrative nature offers multiple potential target sites that may be disrupted through a variety of mechanisms, including endocrine-disrupting mechanisms. Chemical induced effects may be direct, that is, due to an agent or its metabolites acting directly on sites in the nervous system; or indirect, that is, due to agents or metabolites that produce their effects primarily by interacting with sites outside the nervous system. Unless the mechanisms of action are known, it is often difficult to distinguish between direct and indirect effects, even for chemicals that have the known potential to influence hormone action (Chapter 3, section 3.15). Typically, neurobehavioral functions are not directly affected by chemicals but result from chemical-induced morphological and/or functional alterations in a variety of neuroendocrine pathways. This section reviews the extent to which neurobehavioral changes following exposure to neurotoxic chemicals may be related to mechanisms of endocrine disruption. Only neurotoxic chemicals for which effects on endocrine systems may have relevance for neurobehavioral alterations are considered here. These include organochlorine pesticides such as DDT and/or its metabolite DDE, chlordecone (kepone), chlordane, some fungicides (methoxychlor, fenarimol), and, in the PHAH category, the polychlorinated dibenzodioxins (PCDD), polychlorinated/brominated biphenyls (PCB, PBB), and dibenzofurans (PCDFs).

5.2.2 Human Data

5.2.2.1 Developmental neurobehavior.

5.2.2.1.1 PHAHs. The epidemiological literature on neurodevelopmental effects of PCBs published up to 1995 has been reviewed by Schantz (1996). Apart from two mass poisoning events in Japan in 1968 (Yusho) and in Taiwan in 1979 (Yu-Cheng) in which 1,000–2,000 adults were accidentally exposed to high levels of PCBs (and other PHAHs) through contaminated rice oil, there are now four additional cohort studies in which measured PCB concentrations at environmental background levels in relevant body fluids have been related to developmental and neurobehavioral outcomes.

Despite the high levels of exposure (Yu-Cheng mothers: median serum PCB concentrations were 26.8 ng/ml according to Guo et al., 1995a) and the possible contribution of other PHAHs (e.g., PCDFs), both the Yusho and Yu-Cheng episodes, as described by Schantz (1996), provide sufficient neurodevelopmental information for further analysis. In the Yusho incident, apart from the predominant dermal effects (acneform lesions, brown skin pigmentation), other frequent symptoms in affected adults were of central and peripheral nervous system origin and included headache, loss of memory, and hypoesthesia or neuralgia of the limbs. Women who were pregnant when poisoned gave birth to small babies with dark brown skin and other abnormalities. In a subset of children who were followed for several years, persistent growth retardation, movement disorders, generalized slowness, and substantially IQ deficit (average IQ was around 70) were found. In the Yu-Cheng incident the overall picture was similar to the Yusho episode, although the babies born to mothers exposed during pregnancy were followed more carefully over a longer period of time and compared with carefully matched controls (Rogan and Gladen, 1992). A small but systematic lowering of IQ (by one-third of a standard deviation), prolonged p_{300} latencies, and smaller p_{300} amplitudes (p_{300} is a late positive-going component of the event-related brain potential, contingent upon decision/cognitive processes, with latencies of around 300 msec after stimulus onset), as well as evidence of behavioral disorders and increased activity, were reported for the cases relative to the matched controls. However, there was no correlation between the degree of deficit and the PCB levels of the mothers (Schantz, 1996). Because pregnant mothers were advised by their doctors not to breast-feed, effective PCB exposure was most likely prenatal.

Four additional cohort studies, in which measures of internal dose were related to neurobehavioral outcome at different postnatal ages, are currently available; two U.S. studies conducted in Michigan and North Carolina, and two European studies (the Dutch breast milk and the European PCB studies). In the Michigan study, healthy mother–infant pairs were recruited from families with different consumptions of Lake Michigan fish; the other three studies are general population studies. All of them are characterized by background PCB levels measured in different matrices, namely, maternal serum, umbilical cord serum, and/or maternal milk collected shortly after birth. In the Michigan study (Jacobson et al., 1990), out of over 8,000 mothers who had given birth to a healthy child, a total of 313 were recruited for the study who had reported different quantities of fish consumption over the past 6 years and a control group with no self-reported consumption of Lake Michigan fish. An effort was made to measure PCBs in maternal and umbilical cord serum as well as in maternal milk of breast-feeding mothers. PCB-values were available for only about 30% of the cord sera. Mean cord serum levels were 3 ± 2 ng/ml; milk levels were 841 ± 386 ng/g fat (Jacobson and Jacobson, 1996). Neurobehavioral testing took place at several ages between birth and 11 years of age. The overall outcome was as follows (Schantz, 1996): (1) fish consumption but not PCB cord/milk was negatively correlated with motor development, hyporeflexia, and lability at birth; (2) neither fish consumption nor PCB cord/milk was associated with the mental or psychomotor development at 5 months; (3) visual recognition memory was negatively related to PCB cord but not PCB milk at 7 months; (4) at 4 years of age, higher PCB levels in both umbilical cord serum and maternal milk were associated with poorer performance on the McCarthy verbal and numerical memory subtests; and (5) the full-scale and verbal IQ exhibited a negative association with a composite exposure index constructed from PCB/maternal/cord/milk at 11 years (Jacobson and Jacobson, 1996). In summary, postnatal neurobehavioral development appears to be negatively related to *in utero* but not to postnatal PCB exposure.

In the North Carolina study (Gladen et al., 1988), 880 mother–infant pairs were recruited from the general population with more than 700 available for follow-up until 5 years of age. Because PCBs were not detectable in cord serum, they were measured in maternal milk of breast-feeding mothers shortly after birth as well as on later occasions up to 12 months (median at birth, 1.770 ng/g fat;

maximum, 16,000 ng/g fat). Neurobehavioral development of the children was measured at regular 6/12-month intervals after birth up to 5 years of age. Anthropometric measures (height, weight, head circumference) were taken as well. Hyporeflexia and hypotonicity at birth and delayed motor development up to 24 months were associated with prenatal PCB body burden of the mothers indexed by PCBs in early milk samples; no such association was observed beyond 24 months of age. Cognitive development was not affected at any postnatal age. Again, as in the Michigan study, neurodevelopmental delay was taken to be related to *in utero* rather than to postnatal PCB exposure.

In the Dutch breast milk study (Huisman et al., 1995; Koopman-Esseboom et al., 1996), 200 healthy mother–infant pairs were recruited in each of the university hospitals of Groningen and Rotterdam, respectively. Half of the mothers were breast-feeding, the other half formula feeding. Four PCB congeners were measured in maternal and cord plasma and additional PCBs as well as a number of dioxins in early breast milk samples. PCB levels (sum of 118, 138, 153, 180) were as follows: cord plasma, median: 0.43 ng/ml; maternal plasma, median: 2.2 ng/ml; milk, median: 366 ng/g fat. Neurological status and psychomotor and mental development were assessed at 2 weeks, and at 3, 7, and 18 months of age. Additionally, at 7 months, visual recognition memory was measured. Neurological status (hypotonia but not hyporeflexia) exhibited negative associations with PCBs in maternal plasma but not with PCB/dioxins in milk at 2 weeks and 7 months. Psychomotor, but not mental development, was delayed at 3 and 7 months in relation to PCBs in maternal plasma but not cord plasma or milk. At 18 months, the overall neurological status as well as fluency of movements exhibited negative association with PCBs in cord plasma (Huisman et al., 1995). No impairment of visual recognition memory was found to be associated with neonatal PCBs at 3 and 7 months. A follow-up of these two cohorts at 42 months of age was done within the European PCB study described below.

In the European PCB study, in addition to the two Dutch cohorts described above, two additional cohorts of about 170 healthy mother–infant pairs were formed, a Danish cohort from the Faroe Islands and a German cohort from Duesseldorf. The two newly formed cohorts were studied at 2 weeks, 7, and 18 months for neurodevelopment and psychomotor/mental development; the two Dutch cohorts were reassessed for neurodevelopment, language development, and cognitive development at 42 months of age. The common denominator for neonatal PHAH exposure was PCBs in cord and/or maternal plasma as well as in early breast milk samples. PCB levels were as follows (for the Dutch cohorts, see above): for the Faroe Islands cohort, no cord plasma values; milk, 874 ng/g fat (median); for the Duesseldorf cohort, cord plasma, 0.41 ng/ml (median); milk, 405 ng/g fat (median). Results from these studies suggest that (1) mental and motor development between 7 and 42 months of age is negatively associated with PCBs in breast milk but not with cord plasma PCBs, and the degree of association becomes formally significant from 30 months onward (Walkowiak et al., 2001), (2) visual recognition memory does not relate to neonatal PCB at 7 months of age (Winneke et al., 1998), (3) both cognitive development and language development exhibit negative association with PCBs in maternal plasma but not in cord plasma at age 42 months (Patandin et al., 1999), and (4) neurodevelopmental effects are not associated with neonatal PCBs at 42 months of age (Lanting et al., 1998).

In summary, PCBs have been reported to have a negative impact on neurobehavioral development. Delays in postnatal psychomotor or neurological as well as cognitive development have been found to be associated with neonatal PCB exposure (marker congeners 118, 138, 153, 180), although the implicated PCB matrix (maternal vs. cord plasma or early breast milk) differs between studies. The degree of persistence of developmental delay is controversial and the mechanistic basis of such effects is unclear; a hypothyroid mechanism of action (perhaps related to endocrine disruption) of PCBs is discussed below.

5.2.2.1.2 Possible role of thyroid dysfunction. Because of the organizational role of thyroid hormones for development in general and for brain development in particular (Chapter 3), hypothyroid action of PHAHs during development could be a mechanism by which neurobehavioral dysfunction is mediated (Porterfield, 1994). Hypothyroid alterations in association with neonatal PCBs/PHAHs at background environmental levels of exposure have been reported fairly consistently in developmental studies in infants. Within the Dutch breast milk study, negative associations between T_4, T_3 in infant cord plasma, and PCB/PCDD TEQ in maternal (but not cord) plasma, and a positive association with plasma TSH at 2 weeks and 3 months were found (Koopman-Esseboom et al., 1995). Exposure ranges in terms of toxic equivalence factors (TEQ) in human milk were 30.85–154.21 pg TEQ/g fat for total PCB/dioxins. In another Dutch study, TSH elevation together with (unexpected) elevated T_4 was found in association with exposure to PCDF/PCDD in young children; two groups designated "low" (8.7–28 ng dioxin TEQ/kg milk fat) or "high" (29.2–62.7 ng TEQ/kg fat) were compared (Pluim et al., 1993). Also, in a Japanese study, T_4 levels in serum of babies at 1 year of age exhibited negative association with TCDD TEQ (range, 15.2–48.5 ppt, fat basis) measured in maternal milk taken 3 months after birth; other thyroid function parameters were not affected (Nagayama et al., 1997). In all of these studies thyroid hormone levels were in the normal range. As yet it is unclear if such subclinical alterations can be responsible for later neurobehavioral deficit or delay. In one isolated study (Koopman-Esseboom, 1995), no differences were found between neurologically normal (n = 394) and a few abnormal children (n = 23) in terms of thyroid function parameters. However, if exposure during early prenatal life is important, these studies do not give information on thyroid status in the relevant time period.

5.2.2.2 Adult nervous system.

5.2.2.2.1 PHAHs: neurobehavior and thyroid effects. Few studies have examined at neurobehavioral and/or thyroid PHAH effects in adults in relation to occupational or high environmental exposure. Patients from the Yu-Cheng episode exhibited lower nerve conduction velocities and various neurological symptoms, including impaired memory and dullness; PCB levels were 39.3 ± 16.6 ng/ml in blood (Chen et al., 1985). Long-lasting peripheral neuropathy and encephalopathy was found in three cases of PCB-exposed capacitor repair workers exposed for between 4 and 20 years; no data on internal exposure are available (Altenkirch et al., 1996). Firemen exposed to high levels of PCBs mainly judged from the external exposure situation (internal PCB exposure was given in terms of Arochlor 1248 as a median serum PCB level of 6.0 µg/g fat) differed from matched controls in different psychological tests (e.g., short-term memory, visual-motor performance, reaction time); no correlation was found between degree of impairment and individual PCB serum levels (Kilburn et al., 1989). No thyroid effects but a positive correlation between adipose tissue PCBs and 17-hydroxycorticosteroid excretion was found in transformer repair workers (Emmett et al., 1988). Increased thyroid volume but no change in other thyroid function parameters was found in former

employees of a PCB-producing plant or in adolescents living in the polluted neighborhood relative to controls (Langer et al., 1998).

5.2.2.3 Sex hormones and gender-dependent behavior. The role of gonadal hormones during development in affecting behavioral sex differences in humans has recently been reviewed in a comprehensive and hypothesis-guided manner (Collaer and Hines, 1995). In this review, clinical syndromes of hormonal imbalances as well as intentional prenatal exposure to DES and progestins (given to manage at-risk pregnancies) are covered. Girls born to DES-treated mothers appear to be masculinized or defeminized in terms of sexual orientation and language laterality, but not in other respects (core sexual identity, childhood play, cognition). Boys born to DES mothers display less characteristic behavioral changes and evidence on alterations of male behavior. Prenatal exposure of girls to androgen-based progestins was found to be associated with male behavior (e.g., increased tomboyism, higher preference for male-typical toys and male playmates), and more aggression. As for boys, effects, if any, are more subtle, for example, slightly more physical aggression and a tendency to reduced male-typical play. Effects following prenatal exposure to progesterone-based progestins are weak and inconsistent. This information is relevant in the present context, because it can serve as a frame of reference for what may or may not be expected from exposure to environmental low-dose exposure to EDCs in terms of interaction with sex hormones and resulting behavioral alterations. It appears that prenatal exposure to synthetic estrogens (such as DES) or androgen-based progesterone has stronger and more characteristic sexual behavioral effects in girls than in boys.

5.2.2.3.1 PHAHs. Experimentally, interaction of PHAHs with sex steroids and resulting behavioral disruption has clearly been demonstrated in rodents but human data are rare. Some estrogenic-like action of PHAHs has recently been reported (Lanting, 1999). In that study higher volumes of human milk and fat content in breast-feeding mothers was positively associated with maternal PCB burden. Shorter penis length has been reported in connection with the Yu-Cheng incident. Whether or not the behavioral abnormalities in children from this cohort may be interpreted as indicating androgenic or antiestrogenic action of prenatal PHAH exposure needs closer attention.

A gender-dependent behavioral PCB effect was observed in children from the Yu-Cheng cohort. In nonverbal tests for general intelligence based on the solving of maze problems of increasing task difficulty, boys but not girls at 6–9 years of age from the exposed group scored significantly lower than matched controls (Guo et al., 1995b). This suggests of a disturbance of sex hormones due to prenatal PCB/PCDF exposure. In following up the North Carolina cohort until puberty, no evidence of effect, in terms of onset or time course and behavioral manifestations of puberty, was found (Gladen et al., 1996).

5.2.2.3.2 Pesticides. It is known that occupational exposure to pesticides may cause infertility and sterility through hormonal imbalance in males (Strohmer et al., 1993; Straube et al., 1999). Little is known, however, of the possible links, if any, between the known neurotoxicity of many pesticides and any underlying hormonal influence.

Gladen et al. (1988) reported that both PCB and DDE concentrations in maternal milk (median value of 1.77 µg/g fat, and individual values ranging up to 16 µg/g) exhibited an association with abnormal reflexes and hyporeflexia at birth, whereas hypotonicity was only reported for PCBs. It is uncertain if this can be considered a true DDE effect or if it represents a spurious correlation due to the colinearity of PCBs and DDE.

5.2.3 Animal Data

Epidemiological studies on neurobehavioral effects of endocrine disruptors can be corroborated by results of experimental investigations. The focus of this review is on the spectrum of neurobehavioral effects of endocrine-active xenobiotics and sensitive exposure periods. It should be noted, however, that there are many substances for which endocrine effects have been described but that have not yet been examined for neurobehavioral effects.

5.2.3.1 Developmental exposure.

5.2.3.1.1 Sex-dependent neurobehavioral effects. Sex-dependent neurobehavioral effects are responses that are differentially expressed in both sexes but that are not directly related to sexual functions and reproduction. Table 5.6 summarizes studies on such effects following developmental exposure.

Table 5.6 - Neurobehavioral Effects of Developmental Exposure to Endocrine-Effective Compounds in Rats: Sex-Dependent Effects

Compound	Dose and Regimen	Effect	LOAEL	NOAEL	Reference
Chlordane	0.1, 0.5, or 5 mg/kg; GD 4 to PND 80	Improvement of spatial learning (Cincinnati maze) in female offspring	0.1 mg/kg	–	Cassidy et al., 1994
Chlordecone	1 mg/pup on PND 4	Decreased auditory startle responses after a challenge with harmine in adult male offspring, increase in adult female offspring	1 mg/pup	–	Mactutus and Tilson, 1985
Nicotine	0.25 mg/kg/hr by osmotic minipumps; GD 12–20	Elevated sweet preference in adult male offspring	0.25 mg/kg	–	Lichtensteiger and Schlumpf, 1985
PCB 28	8 or 32 mg/kg PO; GD 10–16	Deficit in delayed spatial alternation in female offspring	32 mg/kg	8 mg/kg	Schantz et al., 1995
PCB 118	4 or 16 mg/kg PO; GD 10–16	Deficit in delayed spatial alternation in female offspring	16 mg/kg	4 mg/kg	Schantz et al., 1995
PCB 153	16 or 64 mg/kg PO; GD 10–16	Deficit in delayed spatial alternation in female offspring	64 mg/kg	16 mg/kg	Schantz et al., 1995
PCB 77	1.5 mg/kg SC; GD 7–18	Reduced b-wave amplitudes in the electroretinogram of female offspring	1.5 mg/kg	–	Kremer et al., 1999
PCBs*	40 mg/kg diet, equivalent to 4 mg/kg bw/day, for 50 days before mating until PND 0	Decreased activity of aromatase in the HPOA of male pups at birth; elevated sweet preference in adult male offspring; no pronounced effects of an equal dose of the technical mixture Aroclor 1254 on these end points	4 mg/kg	–	Hany et al., 1999

*Reconstituted mixture (breast milk pattern). PO, per os; SC, subcutaneous injection; bw, body weight.

The elevated sweet preference in male offspring after exposure to nicotine or the reconstituted PCB mixture suggests a behavioral feminization that, for the PCBs, is most likely due to the inhibition of aromatase activity (Chapter 3). Impairments of delayed spatial alternation seen after exposure to single *ortho*-chlorinated PCB congeners suggest changes in the prefrontal cortex that receives a strong dopaminergic projection from the ventral tegmentum. Because dopamine release was shown to depend on estrogen level, this may be the cause for the occurrence of deficits only in females. However, estrogen level and cyclicity were not studied in this experiment (Schantz et al., 1995). The chlordane-induced improvement of spatial learning in females may indicate a masculinization because females perform better on this type of behavior in diestrus when estrogen levels are low. Reasons for sex-dependent changes on auditory startle with chlordecone (Mactutus and Tilson, 1985) and on the electroretinogram with coplanar PCB 77 (Kremer et al., 1999) are not yet known.

5.2.3.1.2 Sexual differentiation and gender-dependent behavior. Effects of endocrine-active compounds on developmental neuronal processes directly related to sexual behavior and reproduction are summarized in Table 5.7. Increased levels of mounting were observed in ovariectomized and testosterone-primed female offspring after chlordecone treatment (LOAEL, 0.5 mg/pup; NOAEL, 0.25 mg/pup; Gray, 1982).

The studies also indicate a demasculinizing effect of TCDD by impairment of malelike sexual behaviors (Mably et al., 1992). In addition, TCDD causes a feminization in males after castration and priming with ovarian steroids. However, some of these effects were expressed less severely in another study of TCDD-induced effects (Gray et al., 1995a, 1995b).

5.2.3.2 Postweaning exposure. Results of neurobehavioral studies devoted to effects on sex-dependent and sexual behavior following exposure to endocrine-active compounds are given in Tables 5.8 and 5.9. In addition to studies in rats, effects of methoxychlor on sexual behavior were also detected in hamsters after 2 weeks of daily exposure to 200 mg/kg orally. Treated ovariectomized females exhibited increased lordosis behavior after priming with progesterone (Gray et al., 1988). Both sex-dependent and sexual behaviors indicate estrogenic effects of methoxychlor. The same holds true for bisphenol A, whereas fenarimol results in demasculinization of males, as indicated by impairment of mounting behavior together with signs of decreased numbers of E_2 receptors in the brain. These effects are most likely due to a developmental inhibition of aromatase activity in the brain (Chapter 3).

5.2.4 Thyroid Hormones

Because of the established role of thyroid hormones in neural development (e.g., neurogenesis, migration of cells in the central nervous system, and cell differentiation), endocrine-active compounds that are assumed to exert neurobehavioral effects by mediation of thyroid hormone–dependent processes have been extensively studied in developing animals. The results are summarized in Table 5.10. In contrast to peripheral type I thyroxine 5′-deiodinase, there is an increase in activity of the type II deiodinase in the central nervous system in response to decreases of circulating T_4 to counteract a decrease in conversion of T_4 to T_3 in the brain. Such activity increases were detected after developmental exposure to single PCB congeners or a technical mixture (Morse et al., 1993, 1996). Another neurobehavioral effect of PCB, for which a mediation by action on thyroid hormones has been demonstrated, is the elevation of hearing thresholds in the low-frequency range; replacement of T_4 during development partially reversed the effects of PCB exposure (Goldey and Crofton, 1998). Although less clear, the same may be true for effects on choline acetyltransferase (Juarez de Ku et al., 1994). For influences on dopaminergic/serotonergic interaction in drug discrimination learning, a similarity of PCB-induced effects and effects by a thyrostatic compound has been shown, but a reversal by T_4 replacement has not yet been demonstrated (Lilienthal et al., 1997).

Table 5.7 - Neurobehavioral Effects of Developmental Exposure to Endocrine-Effective Compounds in Rats: Sexual Differentiation and Behavior

Compound	Dose and Regimen	Effect	LOAEL	NOAEL	Reference
Chlordane	0.1, 0.5, or 5 mg/kg, GD 4 to PND 80	Decreased intromission latencies, elevated number of intromissions prior to ejaculation and elevated number of total intromissions in male offspring	0.1 mg/kg	–	Cassidy et al., 1994
Fenarimol	350 mg/kg diet, equivalent to 35 mg/kg bw, GD 0 to PND 5	Decreased concentrations of nuclear ERs in HPOA of male neonates; reduced concentrations of E_2 and estrone in HPOA of female neonates	35 mg/kg	–	Hirsch et al., 1987a, 1987b
TCDD	64, 160, 400, or 1,000 ng/kg bw PO on GD 15	Demasculinization: prolonged mount, intromission, and ejaculation latencies in male offspring. Feminization: elevated lordosis quotient and lordosis scores in castrated male offspring primed with ovarian steroids	64 ng/kg 160 ng/kg	– 64 ng/kg	Mably et al., 1992
TCDD	700 ng/kg PO on GD 15	Replication of the results by Mably et al. 1992; effects not due to changes in numbers of ERs or in volumes of sexually dimorphic brain nuclei	700 ng/kg	–	Bjerke et al., 1994
TCDD	1,000 ng/kg PO on GD 15	According to cross-fostering, feminized sexual behavior in male offspring due to lactational exposure, effects on demasculinization inconclusive	1,000 ng/kg	–	Bjerke and Peterson, 1994
TCDD	1,000 ng/kg SC, GD 8 or 15	Male sexual behavior less affected or not affected at all by TCDD in contrast to studies by Mably et al. (1992) and Bjerke et al. (1994)	1,000 ng/kg	–	Gray et al., 1995b
TCDD	1,000 ng/kg on GD 15	Vaginal thread in female Long-Evans offspring and difficulties in mating resulting in increased number of mounts without intromissions and elevated ejaculation latencies in unexposed stud males	1,000 ng/kg	–	Gray and Ostby, 1995
TCDD	1,000 ng/kg PO on GD 15	Level of ER mRNA increased in the hypothalamus, uterus, and ovaries and decreased in the pituitary; DNA binding was elevated in the uterus, reduced in the hypothalamus and not altered in the ovaries of peripubertal female offspring	1,000 ng/kg	–	Chaffin et al., 1996
Vinclozolin	200 mg/kg, GD 14 to PND 3	Impaired intromissions and ejaculations in male offspring	200 mg/kg	–	Gray et al., 1994

bw, body weight; PO, per os; SC, subcutaneous injection.

Table 5.8 - Neurobehavioral Effects of Postweaning Exposure to Endocrine-Effective Compounds in Rats: Sex-Dependent Behavior

Compound	Dose and Regimen	Effect	LOAEL	NOAEL	Reference
Methoxychlor	400 mg/kg PO on PND 22	Prior to ovx elevated running wheel activity in treated females and no reduction after ovx in contrast to ovx controls	400 mg/kg	–	Gray et al., 1988
Methoxychlor	200 mg/kg PO from PND 104 throughout testing	After ovx elevated running wheel activity inexposed females that was antagonized by progesterone	200 mg/kg	–	Gray et al., 1988

PO, per os; ovx, ovariectomy.

Table 5.9 - Neurobehavioral Effects of Postweaning Exposure to Endocrine-Effective Compounds in Rats: Sexual Behavior

Compound	Dose and Regimen	Effect	LOAEL	NOAEL	Reference
Bisphenol A	200 mg/kg SC daily for 3 days	Lordosis quotient = 1 in ovx females	200 mg/kg	–	Gray and Ostby, 1998
Fenarimol	4, 8, 17, 35, or 70 mg/kg, PND 21–270	Decreases in number of mounts and increased mount latencies in males	4 mg/kg	–	Gray et al., 1991
Fenarimol 3	50 mg/kg diet, equivalent to 35 mg/kg bw, 7 days	Slightly (not significantly) elevated concentrations of nuclear E_2 receptors in HPOA and pituitary of treated ovx females	35 mg/kg	–	Hirsch et al., 1987a, 1987b
Fenarimol	350 mg/kg diet, equivalent to 35 mg/kg bw, 2 weeks	Nonsignificant reductions by 54% in concentrations of nuclear E_2 receptors in HPOA and pituitary of treated adult males	35 mg/kg	–	Hirsch et al., 1987a, 1987b
Methoxychlor	400 mg/kg PO, PND 22 to adulthood	Lordosis quotient (no. lordosis/no. mounts) = 1 in ovx exposed females after priming with progesterone, while = 0 in primed ovx controls	400 mg/kg	–	Gray and Ostby, 1998
Methoxychlor	200 mg/kg PO, PND 104 throughout testing	Lordosis quotient = 1 in ovx females	200 mg/kg	–	Gray et al., 1988
Methoxychlor	50 or 200 mg/kg PO, PND 21 to adulthood	Increased number of mounts and decreased sperm counts in males	50 mg/kg	–	Gray and Ostby, 1998
Polychlorinated biphenyls	10 mg/kg, daily for 30 days as adults	Reduced percentage of females with sperm in vaginal smear (reduced receptivity?)	10 mg/kg	–	Brezner et al., 1984

bw, body weight; ovx, ovariectomized; PO, per os.

Table 5.10 - Neurobehavioral Effects of Developmental Exposure to Endocrine-Effective Compounds in Rats: Thyroid Hormone–Mediated Effects

Compound	Dose and Regimen	Effect	LOAEL	NOAEL	Reference
PCB 126	250 or 1,000 ng/kg PO, 35 days before mating until PND 21	Elevated hearing thresholds in low-frequency range	250 ng/kg	–	Crofton and Rice, 1999
PCB 77	1 mg/kg SC, GD 7–18	Reduced blocking of apomorphine reaction by buspirone in exposed offspring resembling effects of developmental exposure to a thyrostatic compound	1 mg/kg	–	Lilienthal et al., 1997
PCB 169	0.2, 0.6, or 1.8 mg/kg PO on GD 1	Increased activity of type II T4 5´-deiodinase in whole brain of dams, fetuses (GD 20), and offspring on PND 7 and PND 21	1.8 mg/kg	0.6 mg/kg (no effect at 1.8 mg/kg on GD 20)	Morse et al., 1993
PCB 77 and PCB 169	PCB 77, 1 mg/kg PO, with PCB 169, 0.6 mg/kg, GD 2–18	Increased activity of type II thyroxine 5´-deiodinase in whole brain of fetuses (GD 20), reduced activity in female offspring on PND 21	1 mg/kg PCB 77 + 0.6 mg/kg PCB 169	–	Morse et al., 1993
PCBs, technical mixture (Aroclor 1254)	1, 4, or 8 mg/kg PO, GD 6 to PND 21	Reduced auditory startle amplitudes in offspring on PND 24; permanent elevated hearing thresholds in low-frequency range	4 mg/kg	1 mg/kg	Goldey et al., 1995
PCBs, technical mixture (Aroclor 1254)	1, 4, or 8 mg/kg PO, GD 6 to PND 21	Reduced amplitudes of auditory evoked responses in low-frequency range in offspring	4 mg/kg	1 mg/kg	Herr et al., 1996
PCBs, technical mixture (Aroclor 1254)	8 mg/kg PO, GD 6 to PND 21	Reduced auditory startle amplitudes in offspring on PND 23 and elevated amplitudes in adults; permanent elevated hearing thresholds in low- and high-frequency ranges; reversal of increases in low-, but not high-frequency thresholds by replacement of T_4, no reversal effects on startle amplitudes	8 mg/kg	–	Goldey and Crofton, 1998
PCBs, technical mixture (Aroclor 1254)	62.5, 125, or 250 mg/kg diet, equivalent to 6.25, 12.5, or 25 mg/kg bw, GD 0 to PND 21	Reduced activity of choline acetyltransferase in hippocampus and basal forebrain of exposed offspring, partial amelioration in hippocampus by T_4	6.25 mg/kg	–	Juarez de Ku et al., 1994
PCBs, technical mixture (Aroclor 1254)	5 or 25 mg/kg PO, GD 10–16	Increased activity of type II T_4 5´-deiodinase in forebrain of fetuses (GD 20), reduced activity in female offspring on PND 21	5 mg/kg	–	Morse et al., 1996

bw, body weight; PO, per os.

5.2.5 Conclusions and Recommendations on Neurobehavior

A number of neurobehavioral alterations have been reported to be associated with pre-/neonatal exposure to PHAHs (mainly PCB), although discrepancies exist in terms of the spectrum of effects. Although fairly consistent hypothyroid effects have been found in association with PHAHs in the pre-/neonatal period, a causal role in neurobehavioral dysfunction cannot be deduced from available human data; this is also true for interactions of PHAHs with sex steroids. Biological plausibility is provided by experimental work in animals on some potential endocrine disruptors that indicates exposure-related effects on sex-dependent and sexual behaviors, mediated via sex steroids. In human epidemiological work, measures on these types of behaviors have rarely been included. An exception is a study in Yu-Cheng children, reporting impairments in a special spatially structured intelligence test only in boys. Experimental studies also indicate a thyroid hormone–mediated influence on certain neurobehavioral endpoints, which can be disrupted if exposure occurs during critical periods of development.

Investigations of endocrine disruptors and effects on gonadal steroids and, to a lesser extent, on thyroid hormones have received most of the focus. Possible effects on other hormones due to impaired synthesis, enhanced metabolism, or changes at their targets should also be examined. Gonadal steroids have been implicated in various processes related to neural plasticity, including development, regeneration after injuries, and aging as well as protection against various diseases of the nervous system, neurotoxicants, oxidative stress, and other noxious influences. Endocrine disruptors may alter hormonal actions in all these processes, rendering the nervous system more susceptible to harmful events. Influences on neural plasticity may also impair the ability of adult organisms to adapt to environmental changes. In addition, it is known that the nervous system itself produces the so-called neurosteroids such as pregnenolone, dehydroepiandrosterone, progesterone, and its metabolites, independently of peripheral synthesis. There is no information to date concerning the effect of endocrine-active substances on this spectrum of steroid-mediated effects.

5.3 Immune System

5.3.1 Introduction

5.3.1.1 Outline of structure and function of the immune system. The major function of the immune system is defense against infectious agents and certain neoplastic cells. Various cell types and their soluble mediators execute the function of the system in a finely tuned manner. The host defense can be roughly divided into nonspecific or innate resistance and specific or acquired immunity mediated by lymphocytes (IPCS, 1996).

Components of the immune system are present throughout the body. The lymphocyte compartment is found within lymphoid organs that comprise the bone marrow and thymus, classified as primary or central lymphoid organs, and the secondary or peripheral lymphoid organs that include lymph nodes, spleen, and lymphoid tissue along secretory surfaces, the so-called mucosa-associated lymphoid tissue. Phagocytic cells of the monocyte/macrophage lineage, called the mononuclear phagocyte system, occur in lymphoid organs and also at extranodal sites, such as Kupffer cells in the liver, alveolar macrophages in the lung, mesangial macrophages in the kidney, and glial cells in the brain. Polymorphonuclear leukocytes, which are present mainly in blood and bone marrow and accumulate at sites of inflammation, execute a first line of nonspecific protection.

After initial contact of the host with the pathogen, specific immune responses are induced. The hallmark of this second line of defense is specific recognition of determinants, so-called antigens or epitopes, of the pathogens by receptors on the cell surface of B and T lymphocytes. Following interaction with a specific antigen, the receptor-bearing cell is stimulated to produce a clone of progeny cells that are specific for the eliciting antigen. The specific immune responses help the nonspecific defense presented to the pathogens by stimulating the efficacy of the nonspecific responses. A fundamental characteristic of specific immunity is that memory develops. Secondary contact with the same antigen provokes a faster and more vigorous but well-regulated response.

Two arms of specific immunity are recognized: humoral immunity and cell-mediated or cellular immunity. In humoral immunity, B lymphocytes are stimulated following recognition of antigen by cell-surface receptors. Mature B cells (plasma cells) start the production of antigen-specific immunoglobulins that act as antibodies in serum or along mucosal surfaces. The cellular immunity is mediated by T lymphocytes. They recognize antigen if presented by antigen-presenting cells in the context of histocompatibility antigens. Hence, these cells have a restriction in addition to the antigen specificity. T cells function as helper cells for various (including humoral) immune responses, mediate recruitment of inflammatory cells, and, as cytotoxic T cells, can kill target cells after antigen-specific recognition (reviewed in detail by Schuurman et al., 1991; IPCS, 1996; Weigle, 1997).

5.3.1.2 Immunotoxic responses that could result from EDCs. Toxic responses may occur when the immune system acts as a passive target of chemical insults, leading to altered immune function. Toxicity may also arise when the immune system responds to the antigenic specificity of the chemical as part of a specific immune response, that is, hypersensitivity or allergy. Chemical-induced toxicity, in which the immune system is the target, can result in immunosuppression and potential disease susceptibility, manifested as an increased incidence of infectious disease and certain tumor diseases, as well as the exacerbation of allergic and autoimmune disease (Schuurman et al., 1991; IPCS, 1996, 1999). Although immunotoxicity may occur following exposure to certain EDCs, it is important to distinguish between direct effects on the immune system and effects that are secondary to endocrine disruption. To date, the majority of immunotoxic reactions to chemicals (both EDCs and non-EDCs) where the mechanism is known do not involve endocrine effects. Nevertheless, the immune system and the neuroendocrine system communicate and cooperate closely to maintain physiological homeostasis (Chapter 3, section 3.15). Clearly, there is the potential for chemicals to influence immune function adversely via endocrine mechanisms, and the few known examples of these are discussed later.

Increases in disease in humans, the ultimate outcome of immune dysfunction, are detectable endpoints but causality is difficult to attribute. Thus, for the detection and evaluation of direct immunotoxic effects of chemicals, reliance must be placed on experimental models. A wide array of methods are available to assess immune function. In evaluating the immunotoxicity of chemicals, regulatory authorities in many countries require a multidose 28-day toxicity study in rats. This first screen includes a general set of indicators of the specific and nonspecific immune system, such as are incorporated in OECD guideline 407 (OECD, 1995a). These indicators evaluate toxicity to T- and B-lymphoid cells in primary and secondary lymphoid tissue, as assessed by the weight and the histology of the lymphoid organs. Examination of mucosa-associated

lymphoid tissue is also of value, especially when exposure occurs at the mucosal locations, as is the case in feeding studies. If the screening reveals immunotoxic effects not judged to be secondary to other toxic effects of dhe chemical and the effects occur at a dose level relevant in relation to other toxic effects, second-tier studies are indicated. At the second-tier there is great variety of *in vivo*, *ex vivo*, and *in vitro* assays to assess cell-mediated and humoral immunity, macrophage function, natural killer cell activity, and host resistance in experimental infection models. As understanding of EDCs progresses, it may be necessary to incorporate other tests for immunocompetence into the tiered array.

Host resistance models can be very helpful for risk assessment because they are tools to elucidate the actual consequences of disturbances of immune function. Different host resistance models address different components of the immune system, based on the mechanism in that particular model. Consequently, no single host resistance model can be used as the only tool to evaluate the influence on immunocompetence of exposure to immunotoxic agents (Neumann, 1995; IPCS, 1996).

5.3.2 Human Data

Studies during the last two decades in man and in laboratory animals have clearly shown that the immune system is a target for many compounds, including drugs and chemicals of environmental concern. However, the number of compounds causing immune alterations in humans via a proven endocrine-disrupting mechanism is limited to a few. For DES such a mechanism is clear. For the PCBs, PCDFs, and PCDDs, the toxicity to the thymus, as for most other target organs, appears mediated through AhR binding, resulting in likely effects on thymic hormones. The immunotoxicity may therefore be considered to be mediated through an endocrine-disrupting mechanism. Premature introduction of progesterone has been shown to induce immunosuppression, but the mechanisms reported have been controversial (Siiteri et al., 1977; Schust et al., 1996). A chemical may also show endocrine-disrupting activity but not affect immune function, as reported in the adult rat after perinatal/juvenile exposure to methoxychlor (Chapin et al., 1997a). Thus, for the majority of the few immunotoxic compounds for which the mechanism is known, endocrine disruption is not usually involved.

5.3.2.1 DES. The immunological effects in man and experimental animals following DES exposure have been reviewed by Blair et al. (1992) and Golden et al. (1998). Limited data are available on long-term immune effects in man following *in utero* exposure to DES. Evidence for immunostimulation was reported from increased lymphoproliferative responses to the mitogens PHA and PWM in eight women with reproductive tract abnormalities and evidence of cervical and/or vaginal adenosis (Ways et al., 1987). Another small study of daughters with reproductive tract changes consistent with *in utero* DES exposure suggested possible altered function of natural killer cells (Ford et al., 1983).

Two large DES-exposed cohorts were studied to examine the potential immunological consequences of *in utero* exposure. These studies suggest an increase in the lifetime prevalence of possibly impaired immune function, that is, respiratory tract infections, asthma, arthritis, and lupus, in DES-exposed individuals compared with that observed in the general population (Wingard and Turiel, 1988), or in a control group of nonexposed women participating in the project (Noller et al., 1988). In a follow-up study, using two different groups of DES-exposed women, with an appropriate control group for each, no difference in the prevalence or serum titer of antibodies to five common virus diseases and six less common ones was observed. However, an increased prevalence of a relatively rare immunological hyperreactivity, rheumatic fever, subsequent to microbial infection (strep throat) was found in DES-exposed women (Blair et al., 1992). In a further study, sera of DES-exposed and nonexposed women were examined for the presence of factors associated with autoimmune diseases, and additionally, immunoglobulin levels were determined (Blair, 1992). The incidence of high antibody titers to red blood cell antigen was found to be higher in the DES-exposed females than in the controls and serum IgA values were significantly increased. Blair (1992) concluded that, in general, humans exposed prenatally to DES do not exhibit severe defects in basic immune function, but their propensity to develop autoimmune disease and other diseases associated with defects in immune regulation appears to be increased.

5.3.2.2 PCBs, PCDFs, and PCDDs. For reviews on the effects of PCBs, PCDFs and PCDDs on the immune system, see Birnbaum (1995), Vos et al. (1997/98), and Golden et al. (1998).

5.3.2.2.1 Accidental exposure. Epidemiologic studies on residents of Seveso, Italy, some of whom were exposed to relatively high levels of TCDD, revealed no abnormalities in serum immunoglobulin concentrations and mitogen responses of T and B cells (Reggiani, 1978). Other accidental exposure situations have revealed immunotoxic effects in humans. In a comprehensive examination of people exposed to TCDD-containing waste oils, sprayed for dust control on a dirt road at a mobile home park in Missouri, USA, in 1971, immune alterations were noted. The exposed group had a statistically significant increased frequency of anergy and relative anergy on DTH skin testing and nonstatistically significant, increased frequencies of abnormal T-cell subset test results, a T_4/T_8 ratio of less than 1.0, and an abnormality in the functional T-cell test results (Hoffman et al., 1986). This suggests that long-term TCDD exposure is associated with suppressed cell-mediated immunity, although the question is still open as to whether these alterations were endocrine mediated. In the follow-up of individuals for whom immunologic anergy was shown, none was still anergic. The two most likely explanations for this phenomenon postulated by the authors are the weak potency of the skin test applied in the first study or sensitization to the antigen by the first test (Evans et al., 1988). Another study on the same group seeking clinical correlates with TCDD burden displayed significant increases in both the percentage and absolute number of CD8 T cells and a nonsignificant decrease in the CD4/CD8 ratio. Lymphocyte phenotype analyses in 15 children born to mothers who resided in the mobile home park during and subsequent to pregnancy revealed significantly low frequencies of the T-helper-inducer (CD29/CD4) subset, elevated frequency of the CD8 subset and decreased frequencies of the light-chain-bearing B cells (CD19) (Smoger et al., 1993). Another study of those exposed to TCDD in Missouri suggests an association between TCDD exposure and the activity of the thymic epithelium, with significantly lower mean serum level of the thymic peptide, thymosin-a1 (Stehr-Green et al., 1989). These data support an endocrine-mediated etiology of the immunotoxicity.

Immune alterations have been observed in Taiwanese residents exposed to TCDD-related polyhalogenated aromatic hydrocarbons. Consumption of rice oil accidentally contaminated with PCDFs and PCBs caused acneform skin lesions, pigmentation of skin and nails, liver damage, and abnormal immune function (Yu-Cheng disease). Serum IgM and IgA concentrations and the percentage of

T-lymphocytes in the peripheral blood were decreased (Chang et al., 1981). Investigations using DTH responses showed suppression of cell-mediated immunity (Chang et al., 1982). Children born to female Yu-Cheng patients were also examined. The exposed children had higher incidences of bronchitis during the first 6 months after birth (Rogan et al., 1988) and otitis media at school age (Chao et al., 1997), compared with controls.

As noted above, a disease similar to Yu-Cheng poisoning in Taiwan occurred in Japan in 1968 following exposure to rice oil contaminated with PCBs and PCDFs, the so-called Yusho disease. Yusho patients frequently suffered from respiratory infections. Serum IgA and IgM levels considerably decreased during the 2 years following the onset of poisoning but returned to normal in most cases. Respiratory symptoms persisted for longer time periods (Shigematsu et al., 1978).

Immune alterations were also described in workers exposed to PCDFs and PCBs following a PCB accident in Finland. Investigations showed decrease numbers of T cells in peripheral blood 5 weeks after exposure, with recovery in most cases to normal values 7 weeks later. Lowered T-helper/T-suppressor cell ratios were also observed. During the 7 months after the accident, most of the exposed persons had at least one upper respiratory infection (Elo et al., 1985). A study in workers 17 years after accidental exposure to TCDD showed that antinuclear antibodies and immune complexes were detected significantly more frequently in the blood of TCDD-exposed workers in comparison to matched controls (Jennings et al., 1988).

5.3.2.2.2 Occupational exposure. Occupational studies have shown few immune changes. Veterans of Operation Ranch Hand, the U.S. Air Force unit that sprayed TCDD-containing herbicides in Vietnam from 1962 to 1971, showed no evidence of a consistent relationship between immune system alteration (DTH responses, lymphocyte subpopulations, serum immunoglobulins and autoantibodies) and TCDD exposure category (Michalek et al., 1999). Among workers involved in decontamination work of a chemical plant, with moderately increased body burdens of TCDD and other PCDDs and PCDFs, no relevant alterations in comparison with a control group in peripheral blood lymphocyte subpopulations (Neubert et al., 1993) and lymphoproliferative responses (Neubert et al., 1995). No effects were detected on lymphocyte subsets in the blood or T- and B-cell mitogen-induced lymphoproliferative responses in industrial workers exposed to high doses of TCDD for several years, 20 years prior to testing (Tonn et al., 1996). However, the TCDD-exposed subjects showed a reduced response to human lymphocyte antigen-allogeneic lymphocytes and interleukin 2–boosted proliferation. It was concluded that the long-term immunosuppressive effect on T-helper function is likely mediated by a reduced functionality of individual cells rather than by a reduction in absolute cell numbers in the peripheral blood.

5.3.2.2.3 General population exposure. The effects of breast-feeding versus bottle-feeding and pre- and postnatal exposure on immunological parameters was investigated in healthy infants (the Dutch PCB/dioxin study) from birth to 18 months of age (Weisglas-Kuperus et al., 1995). The total study group consisted of 207 healthy mother–infant pairs, of which 105 infants were breast-fed and 102 children were bottle-fed. Prenatal PCB exposure was estimated by the PCB sum (PCB congeners 118, 138, 153, and 180). Postnatal PCB/dioxin exposure was calculated as a product of the total TEQ level in human milk (17 dioxin and 8 dioxinlike PCB congeners) multiplied by the weeks of breast-feeding. There was no relationship between pre- and postnatal PCB/dioxin exposure and upper or lower respiratory tract symptoms or humoral antibody production. Total and dioxin TEQ levels in breast milk correlated significantly with increased T-cell subpopulations in the infants. Pre- and postnatal exposure to PCBs and dioxins was significantly associated with reduced monocyte and granulocyte counts at 3 months but not at 18 months of age. Additionally, significantly decreased B-cell markers were observed in the breast-fed group following posnatal exposure. In a follow-up study, to investigate whether these changes persisted into later childhood, in preschool children prenatal PCB exposure was associated with increased T-cell numbers, lower antibody levels to measles, and less shortness of breath with wheeze. Current body burden was associated with a higher prevalence of recurrent middle ear infection and chickenpox and a lower prevalence of allergic diseases (Weisglas-Kuperus et al., 1995, 2000).

5.3.3 Experimental and Animal Data

5.3.3.1 DES. As discussed in Chapter 3 (section 3.15), Luster et al. (1984) provided evidence that immunotoxicity of some estrogenic compounds, including DES, correlated for the most part with estrogenicity. Immunotoxicity of exogenous estrogens was mainly manifested as changes in the thymus and thymus-dependent immunity and occurred at pharmacological dose levels, raising the question whether immunotoxic effects can be expected at all with low exposures to weak estrogens. This is in contrast to TCDD, which is a potent immunotoxic compound at low exposure levels (section 5.4.3.2).

The mechanisms responsible for thymotoxicity appear to be mediated through a direct chemical interaction with thymocytes, as well as with nonlymphoid thymic epithelial cells, resulting in the release of soluble immunoregulatory factors by the epithelial cells, probably through binding to ERs or receptorlike structures. From studies performed in laboratory animals, Golden et al. (1998) concluded that they are generally consistent with effects reported in humans exposed *in utero.* Overall, a substantial amount of animal data demonstrate numerous immune alterations following *in utero* exposure to DES, including abnormal B-cell and T-cell responses and diminished natural killer cell activity. The relationship between these effects and neoplasia in rodents is unknown, as is the relevance of these findings to possible health consequences in humans. Most of the immune effects following *in utero* exposure persisted for the lifetime of the animal, and some even became more severe with age. Hence, continued surveillance of humans exposed *in utero* to DES for diseases related to immune dysregulation is warranted.

5.3.3.2 TCDD. Many studies have been performed to investigate the mechanism of TCDD-induced thymic atrophy (reviewed by Vos and Luster, 1989; De Waal et al., 1997). Some indirect mechanisms could be excluded, such as a contribution of stress hormones, because adrenalectomy or hypophysectomy did not influence the TCDD effect. Any role for growth hormone, reduced food intake, or zinc deficiency was also excluded. In mice it was established that the susceptibility to TCDD is genetically determined; susceptibility segregates with the locus encoding a cytosolic receptor protein mediating aryl hydrocarbon hydroxylase activity. This AhR has a high affinity for TCDD, and high levels of this receptor are found in the mouse thymus (of responsive strains) and in the rat thymus. As thymic atrophy is AhR mediated, this response has been used to develop toxic equivalent factors for TCDD congeners (Safe, 1990; Vos et al., 1997/98). In addition,

the splenic plaque-forming cell response to sheep red blood cells, a thymus-dependent humoral immune response that is also AhR mediated, has been used for this purpose.

The effect of TCDD on the thymus may be through an action on epithelial cells, although bone marrow prothymocytes have also been claimed to be targets for TCDD-induced thymus toxicity (De Waal et al., 1997). Thymus atrophy through an effect on epithelial cells is indicated from *in vitro* studies on both cultured mouse and human epithelial cells as well as from *in vivo* data. The enhanced lymphoproliferative capacity of thymocytes after coculture with epithelial cells is reduced when the latter are pretreated with TCDD. These studies also demonstrated that the human thymus may represent a target for the toxic action of TCDD with similar sensitivity to cells of a responsive mouse strain, showing the relevance of mouse data for human risk assessment. A second argument comes from mouse radiation chimeras with TCDD-responsive C57Bl/6 and TCDD-resistant DBA/2 strains, where TCDD-induced suppression of cytotoxic T-lymphocyte activity is determined by the host (epithelium) and not the donor (bone marrow, subsequently thymocytes). Third, TCDD has been shown to act on the thymic epithelium of rats, with formation of epithelial cell aggregates and the appearance of an unusual epithelial cell type and a more differentiated state of the cortical epithelium. Finally, as discussed in section 5.4.2.2.1, investigations of persons exposed to TCDD in Missouri suggest an association between TCDD exposure and the hormone secreting activity of thymic epithelium. Thus, TCDD-induced thymic atrophy may be explained by the inability of epithelial cells to provide the support needed to induce T-cell maturation and differentiation.

Apart from the effect on thymic epithelium, a direct action of TCDD on rat thymocytes has been shown in *in vitro* studies, resulting in a Ca-dependent endonuclease-mediated DNA fragmentation and cell death (apoptosis). However, from *in vivo* studies in which significant thymic atrophy was induced by TCDD, apoptosis appears not the most likely mechanism for *in vivo* thymocyte depletion. A direct action of TCDD on bone marrow stem cells has also been proposed and is substantiated by the finding that bone marrow cells of TCDD-treated donors manifest reduced capacity to populate the thymus of irradiated hosts. In addition to an effect on the thymus and hence on thymus-dependent immunity, TCDD also affects B lymphocytes, as manifested by suppressed antibody responses to thymus-independent antigens. This suppression occurring at higher concentrations, also appears to be AhR mediated.

From the studies discussed above, it can be concluded that TCDD and related compounds cause immune alterations, particularly of thymus-dependent immunity. Clear immuno-suppression with increased rate of infections was noted in the accidental poisoning in the Yu-Cheng and Yusho incidents. Following occupational exposure, effects are limited to one study in which a decrease was reported in T-helper cell function in adult workers as compared with a control group. Results of the Dutch PCB/dioxin study also suggest that background levels influence the human fetal and neonatal immune system, but the functional significance of the latter findings has to be established. The findings in man correlate in qualitative terms with the findings in experimental animals including the sensitivity of the developing immune system, illustrating the relevance of studies in laboratory animals. However, as exposure data on these mixtures of contaminants are mostly lacking for those individuals in which immune parameters were investigated, and because of the remarkable interspecies variation in toxicity of TCDD, quantitative assessment of the likelihood of effects of these chemicals on the human immune system remains difficult. Data that are of relevance in this respect are the above-mentioned studies using thymic epithelial cell cultures showing a similar sensitivity of cells of human and mouse origin. A study by De Heer et al. (1995) investigating the comparative sensitivity of the human thymus to TCDD indicated that the human thymus and the Wistar rat thymus display a similar sensitivity to TCDD, and also suggested that the human immune system is not as susceptible to the immunotoxic effects of TCDD as are very sensitive animal species such as the marmoset, a conclusion also reached by Golden et al. (1998). Regarding the adverse effects of TCDD in general in laboratory animals, immunotoxic effects, both adult and developmental, alongside developmental neurobehavioral (cognitive) effects, developmental reproductive effects (sperm counts, female urogenital malformations), and hormonal effects (endometriosis), were the most sensitive end points on a body burden basis (Van Leeuwen et al., 2000).

5.3.4 Conclusions and Recommendations on the Immune System

Of the large number of compounds with immunotoxic properties, only a few have been shown to cause immunotoxicity that is mediated through an endocrine-disrupting mechanism. These include the potent estrogen-receptor binding DES, which has been shown to cause a weak immunological change following *in utero* exposure, perhaps indicative of potential problems in immune regulation, and the AhR binding PCBs, PCDFs, and PCDDs. Immunotoxicity of DES occurred at pharmacological levels, raising the question of whether immunotoxic effects can be expected at all at low exposure levels to weak estrogens; absence of immune effects from methoxychlor supports this. PCBs, PCDFs, and PCDDs have been reported to alter immune parameters following accidental, occupational, and general population exposures. In particular, the latter findings need further study because they relate to possible effects on immune function from background exposure of the foetal and neonatal immune system. The reported data in humans for DES and for PCBs, PCDFs, and PCDDs are in line with studies in experimental animals. Because for the majority of immunotoxic chemicals, the mechanisms of action are unknown, further investigations are recommended including the study of endocrine-mediated immunotoxicity.

5.4 Cancer

5.4.1 Introduction

Increases in the incidence of certain cancers in many parts of the industrialized world are often cited as evidence that widespread exposure of the general population to EDCs has had adverse impacts on human health. Of particular concern are the observed increased incidences of cancers at hormonally sensitive sites, such as breast, uterus, prostate, and testis in Europe and North America. These increases cannot be adequately explained by improved diagnostic techniques, and it has been argued that these trends coincide roughly with the increasing use and release of industrial chemicals into the environment. Furthermore, these concerns are also based on plausible mechanisms of action because both human and experimental model studies have demonstrated that these cancers are either dependent on or modulated by the hormonal milieu. Under the multistage model of carcinogenesis (Russo and

Russo, 1996), chemicals are thought to act as tumor initiators, as tumor promoters, or as both. In this context, EDCs with estrogenic activity are generally regarded as tumor promoters.

This section focuses on the human data that bear on putative associations between EDCs found in the environment and risk of breast, uterus, prostate, testis, and thyroid cancer. The basic biology of these cancers and the etiologic role of hormones are very complex and beyond the scope of this review. Known risk factors for each cancer site are briefly discussed, emphasizing those factors that could indicate hormonal alterations. The section also integrates the human data with data from animal and experimental model carcinogenicity studies.

5.4.2 Breast Cancer

5.4.2.1 Human data.

5.4.2.1.1 Incidence and risk factors. The incidence of breast cancer increased steadily from the 1940s through the 1990s in many industrialized countries, with the highest risk found in Western Europe and North America. The increases may be due at least in part to increased screening. Relative frequencies vary fivefold among countries. Globally, the lowest rates occur in Asian countries and the highest in the USA and northern Europe (Kelsey and Berstein, 1996). In Japan and China, breast cancer rates are half as high as in the USA (Coleman et al., 1993). When women migrate from one country to another, their breast cancer rates become more similar to that of the host country over a period of two or three generations, suggesting the gradual influence of some environmental or lifestyle factor(s) (Stanford et al., 1995).

Extensive human studies support an etiological role for estrogens in breast cancer, all related to increased lifetime exposure to endogenous estrogen (Hulka and Stark, 1995). These include early age of menarche and/or late onset of menopause; not having children; postmenopausal obesity, which favors the conversion of androgens to estrogens in peripheral fat tissue (Siiteri, 1987); and alcohol consumption which boosts the amount of available E_2 (Reichman et al., 1993). Alternatively, breast cancer risk is markedly reduced by oophorectomy at a young age compared with natural menopause, a phenomenon not seen with other common cancers (Toniolo et al., 1995). The reduced breast cancer risk in Asian women living in Asia is accompanied by 40% reduction in lower serum estrogen levels compared to women in the USA and Great Britain (Key et al., 1990).

It has been suggested that altered reproductive patterns over the last 50 years, including fewer pregnancies and delaying pregnancy until later in life, have significantly increased the number of estrogen surges in modern women, resulting in increased breast cancer risk. In addition to endogenous sources of estrogen, pharmacological HRT and hormonal contraceptives are now commonly used. However, in the case of exogenous hormones, it should be recognized that the issue is not straightforward because for both HRT and oral contraceptives, the types of estrogen that have been used, the doses employed and whether and what type of opposing progestagen has been used are all relevant to the risk.

Hereditary factors also play an important role. A number of genes (e.g., BRCA1, BRCA2) have been associated with increased breast cancer risk (Miki et al., 1994; Lancaster et al., 1996; Li et al., 1997). Prevalence of at-risk genotypes varies widely, and ethnic and geographic differences may be of direct relevance to breast cancer risk. Population-based approaches are just beginning to address the effects of EDCs in high-risk populations carrying breast cancer susceptibility mutations (Ursin et al., 1997; Jernstrom et al., 1999; Brunet et al., 1998). The evidence supports a relationship between breast cancer and hormonal activity.

The U.S. National Toxicology Program has recommended that estrogen be added to the list of "potential human carcinogens." The IARC (1999) summarized studies on combined oral contraceptives and postmenopausal HRT as follows: "There is a small increase in the relative risk for breast cancer in current and recent users, but not for women by 10 years after they ceased using oral contraceptives." The cumulative data on long-term (>5 years) use of combined estrogen/progesterone HRT suggests that this combined therapy increases breast cancer risk (Schairer et al., 2000), but the data remain confusing. Physicians and patients remain faced with having to weigh the risk/benefits of treatment with HRT, contraceptives, or selective ER modulators (e.g., tamoxifen). Risks for endometrial and ovarian cancer are reduced by about 50% with combined oral contraceptive use, reductions persisting at least 10 years after cessation of use. The use of tamoxifen decreases the risk of breast cancer but increases the risk of uterine cancer (van Leeuwen et al., 1994).

5.4.2.1.2 Phytoestrogens. Dietary factors are known to contribute to breast cancer risk (Willett, 2001). One class of dietary compounds that has received much attention is phytoestrogens. Consumption of phytoestrogens, particularly soy products, is higher in Asia than in the Western World (Messina et al., 1994) and has been promoted as having potential anticancer protective effects or as an alternative to HRT. *In vitro* studies with human mammary epithelial (MCF-7) cells, for example, have shown that benzo[*a*]pyrene-induced proliferation is inhibited by genistein treatment and that genistein induces a significant increase in the apoptotic population of cells via a p53-mediated pathway. However, genistein at low concentrations can act as an inhibitor of proliferation in MCF-7 cells, which are estrogen dependent, and in MDA-468 human breast cancer cells, which are estrogen independent, whereas at higher concentrations it acts as an estrogen agonist in MCF-7 cells (Helferich et al., 1998). Other studies suggest that both proliferative and antiproliferative effects might be observed, depending on tumor cell type, dose, timing of phytoestrogen exposure, and phytoestrogen given (Aldercreutz and Mazur, 1997). This may be because phytoestrogens can act via multiple mechanisms of action, both ER mediated and non-receptor mediated, and compounds like genistein have been shown to possess both estrogenic and antiestrogenic properties (Chapter 3). This raises the question of whether foods rich in phytoestrogens may have complex actions in such diseases as breast cancer, exerting both preventive and promoting effects and perhaps depending on whether or not the tumor is estrogen dependent.

There is indirect evidence from both human and laboratory studies that high consumption of soy products may reduce the risk of developing breast cancer (Barnes, 1997). However, the evidence is inconsistent. Soy supplementation in women has been shown to increase the proliferation rate of breast cancer cells in premenopausal women (Petrakis et al., 1996; McMichael-Phillips et al., 1998). However, these studies contain potential confounding factors. Epidemiological studies on the relationship between soy consumption and risk of breast cancer are also inconsistent. A study in Singaporean women found that high soy intake was associated with a lower breast cancer risk among premenopausal but not postmenopausal women (Lee et al., 1991). Studies of Japanese and Australian women with higher concentrations of phytoestrogens in their blood and urine than women in Western countries showed a lower risk of breast cancer (Hirohata et al., 1985). However, a

similar study in women in Shanghai did not find significant differences in soy protein intake between breast cancer cases and their controls (Yuan et al., 1995). Four other studies also suggest that soy consumption is not associated with a reduced breast cancer risk (Hirohata et al., 1985; Messina et al., 1994; Yuan et al., 1995). A meta-analysis of currently available studies indicates that high soy intake might reduce the risk of developing premenopausal breast cancer but has no effect on postmenopausal breast cancer risk (Trock et al., 2000). Thus, caution is necessary in promoting the beneficial effects of phytoestrogens with respect to breast cancer (Bouker and Hilakivi-Clarke, 2000).

5.4.2.1.3 Organochlorine compounds. Although breast cancer is clearly an hormonally related disease, only 30–50% of all breast cancer cases can be attributed to increased life-long exposure to endogenous estrogens (Pike et al., 1993). The unknown etiology of the majority of breast cancer cases, along with large geographic differences in incidence rates, has shifted concerns to the potential role of environmental exposures, particularly exposures to EDCs. The majority of data relating environmental EDCs to human breast cancer are limited to persistent organochlorine compounds that have been identified throughout the world in human tissue, blood, and milk (Adami et al., 1995). Since the mid-1980s, a number of case–control studies have examined the potential association between exposure to organochlorines and breast cancer. These studies have been comprehensively reviewed elsewhere (Adami et al., 1995; Houghton and Ritter, 1995; NRC, 1999) and are therefore only briefly evaluated in this document. Studies published since 1990 are summarized in Table 5.11. The studies cited in Table 5.11 differ widely in terms of number of cases, exposure level, and time of exposure. For details on exposure levels, the reader is referred to the individual references.

DDT. The two main metabolites of DDT, *p,p*-DDE and *o,p*-DDT, have been identified in serum, adipose tissue, and breast milk of individuals with no history of occupational exposure and/or living in areas where DDT has not been used for years. This has raised concern about long-term exposure to DDT through food, which in turn could increase the risk for developing estrogen-dependent tumors such as cancer of the breast.

Around 34 published studies have considered the potential relationship between a nonoccupational exposure to DDT and female breast cancer (Table 5.11). Up to 1993, when Wolff and colleagues published their first study linking DDT exposure to breast cancer, the only available leads had come from four very small and incomplete case–control studies (Wasserman et al., 1976; Unger et al., 1984; Mussalo-Rauhamaa et al., 1990; Falck et al., 1992), and due to design flaws, there is no way to interpret their findings in a consistent manner. More recently, nine prospective case–control studies have failed to demonstrate that DDT, and more specifically *p,p*-DDE, increases the risk for breast cancer (Laden et al., 2001a, 2001b; Ward et al., 2000; Wolff et al., 2000a, 2000b; Hoyer et al., 1998, 2000; Dorgan et al., 1999; Helzlsouer et al., 1999; Hunter et al., 1997; Krieger et al., 1994). In addition, two retrospective case–control studies performed with postmenopausal women (among which breast cancer tends to be more estrogen dependent) did not find increases in breast cancer risk related to DDT exposure (vant'Veer et al., 1997; Moysich et al., 1998), nor did another 10 retrospective case–control studies including pre- and postmenopausal women (Millikan et al., 2000; Demers et al., 2000; Stellman et al., 2000; Aronson et al., 2000; Wolff et al., 2000a, 2000b; Zheng et al., 1999; Bagga et al.,2000; Mendonca et al., 1999; Dello Iacovo et al., 1999; López-Carrillo et al., 1997), in contrast to two positive studies (Romieu et al., 2000; Olaya-Contreras et al., 1998). A recent combined analysis of five U.S. studies showed no relationship between *p,p′*-DDE and breast cancer risk (Laden et al., 2001a, 2001b).

Methodological concerns that might confuse the relationship between DDT exposure and breast cancer were appropriately addressed by most of the recently published epidemiological studies. In general, breast-feeding history was taken into account during statistical analyses as a confounder, because lactation is a way to release the body's DDT burden and also has been shown to be a protective factor for breast cancer (Zheng et al., 1999). Adjustment for lipids in serum to improve serum DDE levels estimation was carried out in most of the recent studies, increasing the reproducibility of the findings and reducing the magnitude of random errors. A third feature has been the stratification by menopausal status or the restriction to postmenopausal breast cancer, to focus on the chance of ascertaining estrogen-dependent tumors, which are more prevalent after menopause.

Another source of concern is the potential existence of a threshold effect for DDE on breast cancer. If true, positive results could be expected from populations exposed to levels of DDT giving rise to serum DDE levels higher than 20,667 ng of DDE, which is the highest level of exposure reported in a nonoccupationally exposed population (Dorgan et al., 1999). However, no evidence of a link between DDT and breast cancer have been found in workers highly exposed to DDT (Cocco et al., 1997).

PCBs. Many of the studies on the relationship between PCB exposure and breast cancer have involved occupational exposure and have not found a positive association (Adami et al., 1995; Houghton and Ritter, 1995; NRC, 1999). In studies on general populations exposed to low levels of PCBs, none have detected a statistically significant increase in breast cancer risk, although at first sight the magnitude of some of the ORs might suggest an effect. The studies summarized in Table 5.11 are difficult to evaluate because PCBs are a mixture of congeners, containing both estrogenic and antiestrogenic compounds. Some studies focused on the concentration of a few individual congeners (e.g., PCB 118, PCB 138; Dorgan et al., 1999; Aronson et al., 2000); others sum the most important peaks of congeners according to their estrogenic potential (Sturgeon et al., 1998) or according to the percentage of chlorinated carbons (Moysich et al., 1998). Another approach has been to sum selected congeners or the total area under the curve and report them as the total amount of PCBs (Hoyer et al., 1998; Hunter et al., 1997; Krieger et al., 1994; Wolff et al., 1993; Moysich et al., 1998; Zheng et al., 1999). Overall, the data do not support an association between PCB exposure and increased breast cancer risk.

DIELDRIN, HCB, AND β-HEXACHLOROCYCLOHEXANE. there are limited data on the association between breast cancer and exposure to dieldrin, HCB, and β-hexachlorocyclohexane (Table 5.11). A study (Hoyer et al., 1998) including 268 cases from a cohort of 7,712 Danish women found a twofold increase in the risk of breast cancer associated with the highest plasma concentration of dieldrin. The relationship to dieldrin also showed a dose response. There were no associations with the other measured 45 compounds (28 of which were congeners of PCBs), and an endocrine mechanism has not been demonstrated.

In a prospective case–control study, after 9.5 years of follow-up, exposure to HCB induced a twofold increased risk of breast cancer among U.S. women diagnosed closer in time to blood study collection but not among women diagnosed later (Dorgan et al., 1999).

Table 5.11 - Summary of Studies on Selected EDCs and Breast Cancer

Reference *Biological Specimen*	Location	Design No. of Cases/ No. of Controls	OR (95% CI) or Mean Difference Cases–Controls (*p*-value)				
			p´p´-DDE	PCBs	β-HCH	HCB	Dieldrin
Laden et al., 2000b, 2001a *Serum*	11 states of USA	Nested case–control 381/381	High vs. low quintile 0.82 (0.49–1.37)	High vs. low quintile Total PCBs 0.84 (0.4–7.3)			
Wolff et al., 2000a, 2000b *Serum*	NYC, USA	Nested case–control 110/213	High vs. low quartile 1.30 (0.51–3.35)	High vs. low quartile Total PCBs 2.02 (0.76–5.37)			
Hoyer et al., 2000 *Serum*	Copenhagen, Denmark	Nested case–control 155/274	High vs. low quartile 1.4 (0.7–2.8)	High vs. low quartile 1.6 (0.8–3.3)	High vs. low tertile 1.2 (0.5–3.0)		
Ward et al., 2000 *Serum*	Norway	Nested case–control 150/150	High vs. low quartile 1.2 (NI)	High vs. low quartile Total PCBs 0.5 (NI)			
Millikan et al., 2000 *Serum*	North Carolina, USA	Case–control 292/270 (African-Americans)	High vs. low tertile 1.41 (0.87–2.29)	High vs. low tertile 1.74 (1.0–3.01)			
		456/389 (Whites)	High vs. low tertile 0.98 (0.67–1.43)	High vs. low tertile 1.03 (0.68–1.56)			
Demers et al., 2000 *Serum*	Quebec, Canada	Case–control 315/307 (population)	High vs. low quintile 1.00 (0.60–1.67)	High vs. low tertile PCB 153 1.28 (0.79–2.19)	High vs. low tertile 0.80 (0.47–1.35)		
		Case–control 315/219 (hospital)	High vs. low quintile 1.36 (0.71–2.63)	High vs. low quartile PCB 153 1.07 (0.54–2.12)	High vs. low tertile 0.83 (0.43–1.61)		
Wolff et al., 2000a, 2000b *Serum*	NYC, USA	Case–control 151/317	High vs. low tertile 0.93 (0.56–1.5)	High vs. low tertile High PCBs: 0.78 (0.45–1.13) Low PCBs: 0.96 (0.53–1.17)			
Stellman et al., 2000 *Adipose tissue*	New York State, USA	Case–control 232/323	High vs. low tertile 0.74 (0.44–1.25)	High vs. low tertile PCB (sum 14 congeners) 1.01 (0.60–1.69)			
Aronson et al., 2000 *Adipose tissue*	Ontario, Canada	Case–control 217/213	High vs. low quartile 1.62 (0.84–3.11)	High vs. low quartile Aroclor 1260 1.15 (0.58–2.25)	High vs. low quartile 0.69 (0.34–1.40)	High vs. low quartile 1.15 (0.57–2.34)	
Zheng et al., 2000 *Adipose tissue*	Connecticut, USA	Case–control 304/186	High vs. low quartile 0.9 (0.5–1.5)				
Romieu et al., 2000 *Serum*	Mexico City	Case–control 120/126	High vs. low quartile 3.81 (1.14–12.80) *p* for trend = 0.02				
Bagga et al., 2000 *Adispose tissue*	California, USA	Case–control 73/73	Per DDE unit 1.12 (0.79–1.6)				
Dorgan et al., 1999 *Serum*	Missouri, USA	Nested case–control 105/207	High vs. low quartile 0.8 (0.4–1.5)	PCB 118: high vs. low tertile ≤2.7 years of diagnosis: 1.4 (0.6–3.2) >2.7 years of diagnosis: 0.9 (0.4–2.4) PCB 138: high vs. low tertile ≤2.7 years of diagnosis: 1.9 (0.8–4.8). *p* for trend = 0.07 >2.7 years of diagnosis: 0.73 (0.3–1.6). *p* for trend = 0.07	High vs. low quartile 0.6 (0.3–1.3)	High vs. low tertile ≤2.7 years of diagnosis: 2.6 (1.1–6.2). *p* for trend = 0.02 >2.7 years of diagnosis: 0.6 (0.2–1.7)	High vs. low quartile 0.7 (0.3–1.3)
Helzlsouer et al., 1999 *Serum*	Washington, USA	Nested case–control 235/235 (1974) 105/105 (1989)	High vs. low quintile 0.10 (0.40–1.32) 0.58 (0.29–1.17)	High vs. low quartile Total PCBs (1974) 1.13 (0.59–2.15) High vs. low tertile Total PCBs (1989) 1.10 (0.38–1.51)			
Dello Iacova et al., 1999 *Serum*	Naples, Italy	Case–control 170/195	High vs. low tertile 1.24 (0.70–2.20)		Mean difference 0.27		
Mendonca et al., 1999 *Serum*	Rio de Janeiro, Brazil	Case–control 177/350	High vs. low quintile 0.83 (0.4–1.6)				
Hoyer et al., 1998 *Serum*	Denmark	Nested case–control 237/469	High vs. low quartile 0.88 (0.56–1.37)	High vs. low quartile Total PCBs 1.11 (0.70–1.77)			High vs. low tertile 2.05 (1.17–3.57) *p* for trend = 0.01

(Continued)

Table 5.11 - *Continued*

Reference *Biological Specimen*	Location	Design No. of Cases/ No. of Controls	OR (95% CI) or Mean Difference Cases–Controls (*p*-value)				
			p´p´-DDE	PCBs	β-HCH	HCB	Dieldrin
Moysich et al., 1998 *Serum*	Western New York, NY, USA	Case–control 154/192	High vs. low tertile 1.34 (0.71–2.55)	High vs. low tertile Total PCBs 1.13 (0.61–2.15) Selected PCBs 1.34 (0.72–2.47)		High vs. low tertile 0.81 (0.43–1.53)	
Olaya-Contreras et al., 1998 *Serum*	Bogota, Colombia	Case–control 153/153	High vs. low quartile 1.95 (1.10–3.52)				
Hunter et al., 1997 *Serum*	Boston, USA	Nested case–control 236/236	High vs. low quintile 0.72 (0.37–1.40)	High vs. low quintile Total PCBs 0.66 (0.32–1.37)			
van't Veer et al., 1997 *Adipose tissue*	Germany, The Netherlands, N. Ireland, Swizerland, Spain	Case–control 265/341	High vs. low quartile 0.48 (0.25–0.95) *p* for trend = 0.02				
Lopez-Carrillo et al., 1997 *Serum*	Mexico City	Case–control 139/139	High vs. low tertile 0.76 (0.41–1.42)				
Krieger et al., 1994 *Serum*	California, USA	Nested case–control 150/150	High vs. low tertile 1.33 (0.68–2.62)	High vs. low tertile Total PCBs 0.94 (0.48–1.84)			
Wolff et al., 1993 *Serum*	New York, NY USA	Nested case–control 58/171	High vs. low quintile 3.68 (1.01–13.5) *p* for trend = 0.04	High vs. low quintile Total PCBs 4.35 (0.9–20.0)			
Stellman et al., 2000 *Adipose tissue*	New York, NY USA	Incomplete case–control 5/5	Mean difference 219	Mean difference 100	Mean difference 2.4	Mean difference 6.1	
Liljegren et al., 1998 *Adipose tissue*	Sweden	Incomplete case–control 43/35	High vs. low tertile 0.4 (0.1–1.2)	High vs. low tertile Total PCBs 0.7 (0.1–2.4)		High vs. low quintile 1.3 (0.3–4.5)	
Guttes et al., 1998 *Adipose tissue*	Germany	Case–control 45/20	Mean difference 62 ($p = 0.01$)	Mean difference PCB 118 = 25 ($p = 0.04$) PCB 153 = 24 ($p = 0.08$)	Mean difference –18 ($p = 0.36$)	Mean difference 18 ($p = 0.40$)	
Schechter et al., 1997 *Serum*	Vietnam	Incomplete case–control 21/21	High vs. low tertile 1.14 (0.23–5.68)				
Sutherland et al., 1996 *Serum*	South Carolina, USA	Incomplete case–control 20/17	Mean difference ER+ 1,366.9 ($p = 0.01$) ER– 156.4 ($p = 0.63$)	Mean difference PCB 99 = 10.2 ($p = 0.05$)			
Dewailly et al., 1994 *Serum*	Quebec, Canada	Incomplete case–control 20/17	Mean difference ER+ 1,366.9 ($p = 0.01$) ER– –156.4 ($p = 0.63$)	Mean difference Total PCBs ER+ 7.7 ($p = 0.79$) ER– –65.5 ($p = 0.39$)	Mean difference ER+ 0 ($p = 0.77$) ER– 5 ($p = 0.92$)	Mean difference ER+ 8.3 ($p = 0.29$) ER– –2.3 ($p = 0.53$)	
Falck et al., 1992 *Adipose tissue*	Connecticut, USA	Incomplete case–control 20/20	Mean difference 703 ($p = 0.04$)	Mean difference Total PCBs 570 ($p = 0.02$)			
Mussalo-Rauhamaa et al., 1990 *Adipose tissue*	Finland	Incomplete case–control 44/33	Mean difference 0.02 ($p = 0.87$)	Mean difference Total PCBs 0.25 ($p = 0.17$)			
Unger et al., 1984 *Adipose tissue*	Denmark	Incomplete case–control 18/35 (autopsies) 14/21 (biopsies)	Mean difference NI (autopsies) 0.02 (biopsies)	Mean difference 1.35 (autopsies) –0.04 (biopsies)			
Wasserman et al., 1976 *Adipose tissue*	São Paulo, Brazil	Incomplete case–control 9/5	Mean difference –3.95	Mean difference∫ 6.15 ($p < 0.01$)	Mean difference 0.22		Mean difference 0.616

NI, no information available.

TCDD AND PBBS. Accidental and/or occupational exposures to the industrial chemical contaminant TCDD and industrial PBB compounds have also been investigated with respect to increased risk of breast cancer. Two studies of women exposed to PBBs in Michigan showed equivocal results (Henderson et al., 1995; Sinks et al., 1996). Women exposed to TCDD in Seveso, Italy, had a decreased risk of breast cancer (Bertazzi et al., 1993), but the number of cases was very small. Two occupational studies on TCDD exposure have shown equivocal results (Manz et al., 1991; Kogevinas et al., 1994).

5.4.2.1.4 Timing of exposure. As noted in Chapter 2, the time of life when exposures take place may be critical to defining the dose–response relationships of EDCs for breast cancer as well as for other health effects. The development of the mammary gland occurs in multiple stages. Fetal development of the mammary gland rudiment is governed by tissue interactions in both males and females. In females, the pubertal period drives ductal morphogenesis, and pregnancy results in massive differentiation of the mammary gland. Thus, the perinatal period and the period between age at menarche and age at first full-term pregnancy may be particularly important for breast tumor development and latency (Snedeker and Di Augustine, 1996). Young girls exposed to carcinogenic agents during puberty may be at high risk of future breast cancer due to susceptibility of rapidly growing breast tissue mediated by hormonal changes during this time. This claim is supported by data from atomic bomb survivors, where an increased risk of breast cancer was found in women exposed before 20 years of age (Tokunaga et al., 1987). Similarly, an elevated risk was found for women irradiated during childhood for medical reasons (Hildreth et al., 1989). Cigarette smoking during prepubertal years may also be related to an increased risk of developing future breast cancers (Palmer et al., 1991). Increased risk was attributed to the age when the girls began smoking rather than the duration of smoking.

The perinatal period may also be a susceptible period for exposures and future breast cancer risk. Some studies have shown that women taking DES during pregnancy to prevent miscarriage have been shown to have a slightly increased risk of developing breast cancer 30 years after taking the drug (Colton and Greenberg, 1993). A recent combined analysis (Titus-Ernstoff et al., 2001) of two cohorts of women demonstrated a modest association between DES exposure and breast cancer risk (risk ratio = 1.27). There was no evidence that DES was associated with risk of ovarian endometrial or other cancers. Data on the risks of breast cancer in daughters of DES-exposed women are not yet available.

Studies of Japanese women with latency periods as long as 35 years found a ninefold greater risk of breast cancer in the subgroup of women who were younger than 4 years of age during the atomic bombing (Tokunaga et al., 1987). Studies in migrants also suggest that the influence of dietary factors in breast cancer is greatest during early childhood and adolescence (Lipworth, 1995).

5.4.2.2 Animal and experimental data. Experimental animal models have provided useful tools for answering specific questions on the biology of mammary cancer relative to their validity on the human disease, as well as for exposure to toxic chemicals (Russo and Russo, 1996). However, there are significant differences in the development of mammary gland neoplasia among species and among strains within a species, and experimental data must be interpreted with caution.

5.4.2.2.1 Synthetic hormones, phytoestrogens, and other estrogens. Experimental studies have clearly shown that hormone treatment can alter mammary gland morphology and tumor sensitivity in rodents and that the observed effects are dose dependent. Studies in female beagle dogs have shown that progestin exposure induces hyperplastic changes in the mammary ductal epithelium (van Garderen et al., 1997) and may increase breast cancer risk (Skegg, 1995). Medroxyprogesterone acetate when administered to rodents, cats, and monkeys, alone or in combination with estrogens, results in an increased risk of mammary cancer development (Rutteman, 1992; Skegg, 1995). Tamoxifen has been shown to protect against mammary gland tumors in various strains of rats (Jordan, 1991; Kotoula et al., 1993; Ménard et al., 2000).

As noted above, phytoestrogens can have a variety of endocrine-modulating effects and properties (Barnes, 1998a, 1998b). Experimental studies support the human observations that timing of exposure may be the critical factor in determining the effects of phytoestrogens. For example, isoflavones found in soy products have been generally thought to confer reduced breast cancer risk. In seven out of nine animal studies, a lower number of tumors were observed in rats whose diet was supplemented by soy (Barnes, 1997). However, genistein administration in rats during gestation results in a dose-dependent increase in mammary tumor susceptibility in the F_1 animal (Hilakivi-Clarke et al., 1999a, 1999b). Immature rats given subcutaneous injection or infusion of the estrogenic compound bisphenol A show mammary gland proliferation (Colerangle and Roy, 1997).

Experimental models also offer tools to assess the interaction between genetic factors and environmental exposures on breast cancer risk. *In vivo* studies, using mice heterozygous for the breast cancer susceptibility genes, BRCA1 and BRCA2, showed that exposure to DES for 26 weeks (beginning a week before ductal morphogenesis) led to inhibited growth and differentiation of ducts, suggesting compromised DNA repair processes (Bennett et al., 2000).

5.4.2.2.2 Organochlorine compounds and genotoxic agents. The evaluation of chemicals in 2-year bioassays in laboratory rodents has become the cornerstone for identifying those chemicals most likely to cause cancer in humans (Huff et al., 1991a, 1991b). However, these carcinogenesis assays do not include exposure during pregnancy and lactation that can influence expression of mammary gland carcinogenesis (Grubbs et al., 1985). The mammary gland is a frequent source of tumors or neoplasms in laboratory rodents. They may occur spontaneously and are also induced by genotoxic agents. The long latency and variable incidence of mammary tumors in rodent strains limit the utility of studying such tumors as models of human disease (Neumann et al., 1996). Genotoxic agents such as dimethylbenz(*a*)anthracene and 3-methylcholanthrene are commonly used to elicit mammary gland tumors in rodents. These carcinogenic agents are thought to act as initiators within the context of a multistage model of carcinogenesis (Russo and Russo, 1996). Following initiation with such genotoxic agents, hormonal factors may promote tumor formation. Many of these chemicals have not been strongly linked to breast cancer in epidemiological studies.

Although there has been suspicion about involvement of organochlorine compounds in human breast cancer development, these compounds have not generally induced mammary cancer in animals. Chemicals measured include pesticides—DDT and its major metabolite DDE, chlordane, HCB, benzene hexachloride (or lindane), and halogenated biphenyls—as well as PCBs, PBBs, and TCDD. With the exception of DDT, none of these compounds has been associated with mammary gland cancer in animals, although other site-specific cancers have been identified (Wolff et al., 1996).

The triazine herbicide, atrazine, has been reported to promote mammary tumor growth in a certain strain of rats, but the mechanism of this response is not relevant to breast cancer in humans (Chapter 3, section 3.13).

5.4.2.3 Conclusions and recommendations on breast cancer. Although numerous human epidemiological studies have been conducted to determine whether environmental EDCs may contribute to an increased risk of breast cancer, the results remain inconclusive. Overall, the current scientific evidence (from human and animal studies) do not support a direct association between exposure to environmental EDCs and increased risk of breast cancer. However, all the studies published to date have measured EDC exposure levels in adult women. The claim that the time of life when exposure takes place (e.g., prenatal, neonatal, childhood, adolescence) may be the most critical factor is supported by human data on radiation and smoking and by basic research in animal models. Adult women currently at risk for breast cancer may have been exposed to exogenous EDCs *in utero* or during infancy, childhood, and adolescence in the mid-1900s when contaminant levels of organochlorines were higher. Research is urgently needed to address the role of timing of exposure. Because human prospective studies would be complex, time-consuming, and expensive, researchers should be encouraged to utilize and develop animal models to address this important issue and to use serum banks from this time and conduct retrospective follow-up studies.

Breast cancer is likely due to many factors, including genetics, lifestyle, diet, endogenous hormone status, and environmental factors. Research on whether potential complex interactions among these factors, modulated by individual genetic susceptibility factors, produce breast cancer is critical. Until consistent and compelling data on these issues become available, the role of EDCs in contributing to breast cancer incidence is likely to remain a highly controversial issue.

5.4.3 Endometrial Cancer

5.4.3.1 Human data. The uterus is highly responsive to hormonal alterations. Cancer of the uterus is more common in developed countries, with a similar pattern of hormonal risk factors as breast cancer. There is clear evidence that unopposed estrogen is the major risk factor for endometrial cancer (IARC, 1999; Potischman et al., 1996). However, there is no increasing temporal trend for endometrial cancer.

Epidemiologic data on the effects of environmental EDCs on endometrial cancer are limited. Sturgeon et al. (1998) found no association between endometrial cancer and 27 PCB congeners, 4 DDT-related compounds, and 13 other organochlorine compounds. Several retrospective occupational cohort studies also observed no association (Bertazzi et al., 1987; Brown, 1987; Sinks et al., 1996). In the Seveso industrial accident, TCDD exposure appeared to reduce the risk of uterine cancer, but the number of cases was small (Bertazzi et al., 1993).

There is some evidence that dietary isoflavones protect from endometrial proliferation, as shown by a decreased incidence of endometrial cancer in Japanese and U.S. (Hawaiian) women consuming isoflavone-rich diets. Specifically, high consumption of soy products and other legumes was associated with a decreased risk of endometrial cancer for the highest compared with the lowest quartile of soy intake (p for trend = 0.01; OR, 0.46; 95% CI, 0.26–0.83).

5.4.3.2 Experimental and animal data. Numerous studies have characterized the estrogenic potential of environmental chemicals using *in vitro* endometrial cancer cell model systems or *in vivo* classical uterotropic assay (Klotz et al., 1997; Hunter et al., 1999). Certain phytoestrogens, such as genistein and daidzein (Santell et al., 1997; Boetger-Tong, 1998), and some environmental chemicals (e.g., methoxychlor, nonylphenol, bisphenol A) have all been shown to induce a uterotropic response in rodents (Odum et al., 1997; Ashby, 1998). Neonatal treatment of mice on PNDs 1–5 with either DES or the phytoestrogen, genistein, has been shown to cause uterine adenocarcinoma by 18 months (Newbold et al., 2001). Though others have shown that soy isoflavones inhibit E_2-mediated endometrial proliferation in macaque monkeys (Cline and Foth, 1998), which is consistent with the human study mentioned above. This is perhaps not surprising in the case of phytoestrogens that have both estrogenic and antiestrogenic effects (Whitten and Patisaul, 2001).

However, as discussed in Chapter 3, there are few data on whether long-term exposure to these chemicals at low doses can result in neoplasms. Long-term exposure in rats to toxaphene (Reuber, 1979) and methoxychlor (Reuber, 1980) resulted in uterine hyperplasias. Exposure to the antiandrogenic herbicides (atrazine and vinclozolin) at high doses increased uterine adenocarcinoma development in rats (IARC, 1991; Mellert, 1995).

As with other cancers, the timing of exposure is critical to the potential development of uterine cancer. Developmental exposure of mice to DES results in uterine neoplasms, but treatment of adult mice with comparable levels of DES does not induce uterine neoplasms (Newbold et al., 1991).

5.4.3.3 Conclusions and recommendations on endometrial cancer. Because endometrial tissue is very responsive to the actions of antiestrogenic and estrogenic compounds, it should be a sensitive target tissue for EDC action. However, neither limited human data nor animal studies currently support an association between exposure to organochlorines and risk of endometrial cancer.

5.4.4 Testicular Cancer

5.4.4.1 Human data. Testicular cancer is the most common malignancy among young men, 25–34 years old (Adami et al., 1994). Most of the tumors occurring in young men are seminomas of germ cell origin; hence, early exposures may be relevant. Toppari et al. (1995) have estimated that testicular cancer incidence in men under 50 has increased 2–4% per annum since the 1960s in many developed countries, and in countries with a long history of cancer registration, the start of the increase can be traced back to around 1920 (Bergstrom et al., 1996). There are marked differences in incidence levels of testicular cancer between countries and between races. The incidence in Denmark is about fourfold higher than in neighboring Finland, which parallels observations of higher sperm counts, a low incidence rate for hypospadias, and lack of temporal increase in hypospadias in Finland (sections 5.1.3.1 and 5.1.6.4). In the USA, Caucasians have about a threefold higher incidence than do African Americans. Cryptorchidism (section 5.1.7) is a known risk factor for testicular cancer, suggesting a possible prenatal etiology (Moss et al., 1986) or, as some have suggested, there may be modulation by early postnatal exposure to estrogens or antiandrogens (Bergstrom et al., 1996; Ekbom et al., 1996; Moller and Skakkebaek, 1999).

No single study of sons of DES mothers has shown a significant increase in testicular cancer, but a meta-analysis of available studies concluded there was an overall increase of approximately twofold, which was just statistically significant (Toppari et al., 1996).

There are currently no published epidemiological studies of testicular cancer in which blood concentrations of environmental

EDCs have been measured. A recent regression analysis (Cocco and Benichou, 1998) used *p,p*-DDE concentrations from human adipose tissues obtained in 1968 to predict testicular cancer among white males in 22 U.S. states, found no association between the antiandrogen DDE and testicular cancer some 2–22 years later.

5.4.4.2 Experimental data. The type of testicular cancer commonly found in humans, seminomas, which are preceded by atypical intratubular germ cells termed CIS, is extremely rare in laboratory animals. The testicular tumors in rodents induced by some chemicals are Leydig cell tumors (Cook et al., 1999). Thus, until recently, experimental animal models appropriate for extrapolation to humans were lacking. However, atypical germ cells resembling human CIS cells have now been reported in cryptorchid stallions (Veeramachaneni and Sawyer, 1998; Veeramachaneni, 2000) and in an infertile rabbit (Veeramachaneni and VandeWoude, 1999), in association with developing tubular seminomas. In further studies, similar CIS lesions have been induced in rabbits exposed in utero and/or in infancy to the EDCs octylphenol, *p,p′*–DDT/DDE or zeranol (Veeramachaneni, 2000). These treatments also resulted in a variable incidence of undescended testes with CIS-like cells in both undescended testes and scrotal testes, but surgically induced cryptorchidism did not result in CIS, showing that the germ cell atypia was induced by the chemical exposure and not by abdominal retention of the testes. *In utero* exposure to dibutyl phthalate in the rabbit has also been shown to result in undescended testes, ambiguous genitalia, hypospadias, regressed prostate, and missing bulbourethral glands (Higuchi et al., 1999), analogous to effects seen following treatment with phthalates in the rat (Chapter 3). These observations are relevant because cryptorchidism is a known risk factor for CIS in humans, and they illustrate the potential relevance of the rabbit as a model for this type of cancer in humans.

5.4.4.3 Conclusions and recommendations on testicular cancer. Risk factors for testicular cancer are associated with disorders of androgen production or action. There are also limited data from animal studies that exposure of the male fetus to high levels of estrogen may increase the risk of developing testicular cancer. However, there are no published analytical epidemiologic studies that examine a connection between exposure to estrogenic and/or antiandrogenic (e.g., DDE) compounds and testicular cancer. Moreover, validated animal models for germ cell (testicular) tumors observed in man currently do not exist. Increased research efforts are needed to develop suitable animal models for testicular cancer, so that the effects of prenatal and postnatal exposure to EDCs can be investigated. Although the data are limited, some evidence suggests that the incidence of cryptorchidism and hypospadias may show similar geographic differences to the incidence of testicular cancer. The potential roles of other environmental factors (e.g., diet, occupational exposures) in producing testicular cancer are unknown and need to be investigated.

Efforts to obtain data regarding the possible existence of a testicular dysgenesis syndrome, of which reduced sperm quality, cryptorchidism, and testicular cancer are components, are also needed, along with research on possible shared etiologies.

5.4.5 Prostate Cancer

5.4.5.1 Human data. Prostate cancer is the most commonly diagnosed cancer among men in developed countries (Parker et al., 1997). Since the mid-1980s, age-adjusted incidence rates have increased abruptly, which can be largely attributed to improved screening and diagnostic tests. However, what accounts for the long-term rise in both incidence and mortality is not known. There are racial differences in susceptibility, with the incidence being rare in Asians, 20–30 times higher in Caucasians, and even higher in African-American males (Crisp et al., 1998).

Little is known about the causes of prostate cancer, but it is both hormone dependent and able to be modulated by hormone treatment (section 5.1.6.5). A few small epidemiological studies on men in Japan and Japanese migrants to the USA indicated a tendency for increased soy consumption to be associated with lower prostate cancer risk (Morton et al., 1997). The limited epidemiologic data on potential associations between prostate cancer and exposure to environmental EDCs are derived mainly from occupational exposures, and all of them lack internal exposure information. Occupational studies of PCB-exposed workers have not shown an association between PCBs and prostate cancer (Bertazzi et al., 1987; Brown, 1987; Sinks et al., 1992). No significant increases of prostate cancer were reported as a result of accidental TCDD exposure in Seveso, Italy (Bertazzi et al., 1993). Similarly, in the cohort study based on the international registry of workers exposed to TCDD, there was no increased prostate cancer mortality (Saracci et al., 1991). Other studies on workers in Germany (Becher et al., 1996) and the USA (Fingerhut et al., 1991), showed a small but statistically insignificant excess in prostate cancer mortality, based on a limited number of cases. The regression analysis (Cocco and Benichou, 1998) using DDE concentrations of adipose tissues (obtained in 1998) also indicated no positive association between DDE and prostate cancer mortality. In a retrospective cohort epidemiology study of Canadian farmers linked to the Canadian National Mortality Database, a weak but statistically significant association between acres sprayed with herbicides and prostate cancer deaths was found (Morrison et al., 1993). However, the possible role of chemical exposure and endocrine disruption as a contributing factor in the etiology of adenocarcinoma of the prostate cannot be excluded.

5.4.5.2 Experimental data. Experimental research on the etiology of prostate cancer has been hindered by the lack of suitable animal models for study (Bosland, 1992). Rodent models are of uncertain relevance to man. In contrast to its frequent occurrence in humans, prostate cancer is rare in laboratory rodents, but the frequency can increase significantly with hormone treatment depending on the strain of rat, dose of hormone, and duration of treatment (Bosland, 1992). Dogs can develop prostate cancer, but further research is needed to determine its relevance to humans. There are few experimental studies on the effects of phytoestrogens on prostate cancer. In three animal studies, the effects of soy showed reduced tumorigenesis (Lee et al., 1991; Messina et al., 1994). Genistein has been shown to inhibit chemically induced prostate cancer in rats (Pollard et al., 2000). The green tea polyphenol EGCG is known to rapidly reduce the size of human prostate and breast tumors grown in nude mice, and its been hypothesized that consumption of green tea may contribute to the lower mortality from these tumors in some Asian countries. A recent study showed that EGCG may inhibit androgen action by repressing transcription of the AR gene (Ren et al., 2000). Very few chemicals have been identified that can induce prostate cancer in the 2-year bioassay studies (Huff et al., 1991a, 1991b). Transgenic mouse models for prostate cancer have been developed and offer opportunities to study hormone-responsive elements and the effects of chemicals on the multistage progression of prostate cancer (Gingrich et al., 1996). Future studies using these models may provide additional information on the etiology of prostate cancer.

5.4.5.3 Conclusions and recommendations on prostate cancer. It is known from experimental data (section 5.1.6.5.1) that the development of the prostate gland or the propensity of this organ to develop cancer can be affected by perinatal/postnatal exposure to estrogens and phytoestrogens and possibly also androgens and AhRs. The few epidemiologic studies on prostate cancer do not include measurements of dose in body fluids or tissues. Studies on PCB, TCDD, and DDT exposures showed no association with increased prostate cancer. Exposure to herbicides or polyaromatic hydrocarbons has been linked to prostate cancer, but the evidence is weak, the mechanism is unknown, and more research is needed. Little is known about the effects of other environmental factors (e.g., genetics, diet, endocrine status) on the incidence of prostate cancer.

5.4.6 Thyroid Cancer

5.4.6.1 Human data. As mentioned in Chapter 3 and section 5.3, the thyroid gland plays a key role in numerous endocrine and metabolic and physiological functions. The thyroid hormones are particularly important to processes involving growth and development and some environmental chemicals (e.g., certain PCBs) have been shown to possess antithyroidal activity (Porterfield and Hendry, 1998). Thyroid hormones are also involved in the carcinogenic process and can affect tumor formation, growth, and metastasis (Guernsey and Fisher, 1990). Thyroid cancer is an uncommon and largely nonfatal tumor with incidence rates two to three times higher in females than in males (Landi et al., 1998). Nordic countries appear to have the highest incidence rates (Coleman et al., 1993). In contrast to clinically apparent disease, small occult thyroid tumors are noted at autopsy in up to about 50% of cases surveyed. The only known human thyroid carcinogens are x-rays and ionizing radiation (NRC, 1990; Lomat et al., 1997). Persons living in iodide-deficient areas of the world are unable to synthesize adequate levels of thyroid hormones and develop hyperplastic thyroid lesions. There is conflicting evidence whether thyroid cancer is increased in these individuals (Galanti et al., 1995). In epidemiologic studies, goiter and thyroid nodules have been shown to be risk factors for thyroid cancer (Ron et al., 1987). Graves' disease and Hashimoto's disease often precede thyroid carcinoma and may be part of the causal pathway. There is some evidence that sustained stimulation of TSH receptors is important for development of thyroid cancer in chronic goiter cases (Shi et al., 1991). To date, no environmental chemical has been identified as being carcinogenic to the human thyroid. The etiology of thyroid cancer in humans is largely unknown, and limited trend data are available.

5.4.6.2 Experimental data. Rodents and humans share a common physiology in regard to the hypothalamic-pituitary-thyroid feedback system, and the thyroid is a commonly affected target organ in rodent chemical carcinogenicity studies (Huff et al., 1991a, 1991b). In a review of potential carcinogenicity of 240 pesticides, at least 24 (10%) produced thyroid follicular cell tumors in rodents (Hurley et al., 1998). Mutagenicity does not appear to be a major determinant in thyroid carcinogenicity for pesticides (except for possibly acetochlor), in contrast to some other chemicals, such as aromatic amines (Hill et al., 1989). The mechanism of action by nongenotoxic agents is thought to be due to a sustained increase in serum TSH levels (Kanno et al., 1996), which can occur through various perturbations of the hypothalamic-pituitary-thyroid axis (section 3.5). The most potent thyroid carcinogens are TPO inhibitors, which can cause a drastic decrease in serum thyroid hormone levels and trigger TSH hypersecretion from the pituitary gland via release from negative feedback. Highly potent TPOs are thionamides (thiourea, ethylene thiourea, propylthiouracil, etc.) and aminotriazole (Hill et al., 1989). It is also postulated that during these reactions, free radicals are generated that interfere with other enzymes and bind to other proteins and possibly to DNA (Krauss and Eling, 1987). Other chemicals (e.g., the pesticides clofenzetine, fenbuconazole, pentachoronitrobenzene) appear to enhance the hepatic metabolism and excretion of thyroid hormones.

The environmental chemicals TCDD, PCBs, and PBBs also increase the metabolism of thyroid hormones, resulting in potential increases in thyroid neoplasms (Barter and Klaassen, 1992). PCBs have also been shown to block the binding sites for T_4 to serum transport proteins that causes enhanced clearance from serum and decreased availability to tissues (Brouwer and Van den Berg, 1986). PCBs do not bind directly to the thyroid receptor (Cheek et al., 1999).

5.4.6.3 Conclusions and recommendations on thyroid cancer. A direct association between exposure to specific EDCs and thyroid cancer is not supported by human experimental data. However, some EDC chemicals can affect the hypothalamic-pituitary-thyroid axis, and the basic mechanisms of interaction among various hormonal systems need to be elucidated to understand the process of thyroid carcinogenesis in humans.

5.4.7 Conclusions and Recommendations on Cancer

Although there is biological plausibility and some experimental evidence that EDCs may contribute to hormonally influenced human cancer, the current state of the science has not provided clear evidence for a causal link. In the case of testis cancer, human studies have not yet explored this possible link. Where possible associations with EDC exposure have been explored (mainly for breast cancer), the overall strength of the evidence of a causal association is weak. However, there is not enough information to completely reject the hypothesis that endocrine disruptors such as PCBs, dieldrin, or some other not yet evaluated compound(s) could play a role in the incidence of (female and/or male) breast, endometrial, prostatic, and testicular malignant tumors. Further research should focus on the assessment of exposure to endocrine disruptors during critical periods of human development (intrauterine life, adolescence, etc.), in relation to the occurrence of cancer at endocrine-sensitive sites during childhood or at later stages of life. Cancer registries will continue to provide useful information on geographical and temporal trends in cancer incidence that could be exploited for hypothesis testing.

5.5 Other Endocrine Systems Potentially Vulnerable to EDCs

In this chapter, the main focus has been on the reproduction, central nervous and immune systems, and cancer at endocrine sites. It is clear, however, that endocrine disruption in its broadest sense encompasses more than just these targets and may involve hormones other than sex and thyroid hormones. The potential effects of EDCs on other hormones and their target organs, such as growth hormone, insulin, and adrenocortical hormones, are not reviewed in detail here because the available research to date is very limited. The physiological roles of some of these other hormones are outlined in Chapter 3, indicating the scope for adverse effects should these systems prove vulnerable to EDCs. As an illustration of exploratory work underway on other endocrine systems, a brief review of glucocorticoids is given below.

The developmental and functional endocrinology of the hypothalamic-pituitary-adrenal axis is described in Chapter 3 (section 3.4). Glucocorticoids bind to intracellular GRs, which belong to the same receptor family as the sex hormone receptors. The similarities between these two systems are many, for example, with respect to regulation of synthesis and the functioning of the receptors, indicating that the glucocorticoid system also may be vulnerable to disturbance by xenobiotics. Most cells contain GR, but they are especially abundant in target organs such as the liver, the hypothalamus, the pituitary, and the hippocampus. Given the many functions of glucocorticoids in the body (metabolic, cardiovascular, developmental, immunosuppressive, anti-inflammatory), the potential for interference by EDCs is evident. In normal fetal development, for example, glucocorticoids are responsible *inter alia* for pulmonary surfactant secretion and the regulation/differentiation of neural crest cells of the nervous system. Excessive and prolonged exposure to glucocorticoids, such as during chronic stress, has recently been shown to induce neurodegeneration in the hippocampus, a brain structure involved in learning and memory processes; similarly, effects on behavior are observed in GR knockout mice (De Kloet at al., 1998, Sapolsky, 1996).

Experimental studies have indicated several mechanisms whereby xenobiotics can interfere with glucocorticoid homeostasis. The best known is the cytotoxicity exerted by the DDT metabolite DDD in adrenocortical cells (Nelson and Woodard, 1949; Adamson et al., 1973); *o,p′*-DDD has therefore been used in the past as a drug to suppress cortisol secretion. Methylsulfonyl-DDE is another DDT metabolite that is adrenotoxic (Lund et al., 1988). In mice, a single administration of 12 mg/kg body weight results in a decreased capacity to synthesize corticosterone that remains for at least 40 days (Jönsson, 1994). The mechanism involves bioactivation by CYP11b1 (a mitochondrial enzyme only present in the adrenal cortex), mitochondrial damage, and finally cytotoxicity (Lund and Lund, 1995; Jönsson et al., 1991).

Inhibition of adrenocortical enzymes may also result in disturbed glucocorticoid homeostasis. Azole compounds, discussed above in relation to inhibition of cytochromes involved in synthesis of sex hormones (e.g., aromatase inhibitors; see Chapter 3, sections 3.12.5.2 and 3.12.5.3), are also likely to affect the glucocorticoid pathway. Several pharmaceuticals (e.g., ketoconazole and metyrapone) have been shown to be potent inhibitors of glucocorticoid synthesis (Couch et al., 1987). There is also scope for interactions between the glucocorticoid and sex hormone synthesis pathways because the first steps are identical, involving transformation of cholesterol via (17-hydroxy-)pregnenolone into (17-hydroxy-)progesterone. There are data indicating that inhibition of adrenocortical enzymes mediating the further metabolism of (17-hydroxy-)progesterone may lead to an increased formation of sex hormones. Mechanistic *in vitro* studies have shown that a shift from glucocorticoid to androgen production is caused by the increased availability of substrate for the production of sex hormones (Mesiano et al., 1999). Thus, the adrenocortical production of androstenedione and dehydroepiandrosterone is normally rather insignificant but may become important in pathological conditions and at different life stages, such as at adrenarche (Papadimas, 1997).

Finally, xenobiotics may also interfere with glucocorticoid homeostasis by interacting with the GR. Studies have shown that xenobiotics may both up- and down-regulate the density of GR in organs (Budziszewska et al., 1995; Bellingham, 1992). Recent *in vitro* data also suggest that xenobiotics may bind directly to the GR. Johansson et al. (1998) studied 24 ubiquitous methylsylfonyl-PCBs and found that, at micromolar concentrations, some methylsylfonyl-PCBs compete with dexamethasone in binding to the GR. One of them was studied in Chinese hamster ovary cells carrying the human GR and a reporter gene, which indicated an antagonistic effect of 3-methylsulfonyl-2,5,6,2′,4′,5′-hexachlorobiphenyl at the GR.

These observations in experimental models are supported by wildlife observations, which indicate that perturbations of the glucocorticoid system do occur. Canadian studies have consistently shown that fish from polluted waters have a decreased capacity to synthesize cortisol in response to stress (Hontela, 1998). Lorentzon et al. (1999) have recently shown that current levels of organochlorines in herring gull (*Larus argentatus*) embryos are inversely related to blood corticosterone concentration and activities of corticosterone-dependent intermediary metabolic enzymes. The finding that DDT and corticosterone induce similar pathological development of the upper mandible in tadpoles has raised the question of whether DDT mimics corticosterone or acts via corticosterone by inducing stress that subsequently increases the levels of corticosterone (Hayes et al., 1997).

Given the above findings in laboratory animals and in wildlife, it is clear that there is potential for effects on glucocorticoid homeostasis from certain EDCs such as DDT and PCBs. As yet, there are no human studies on exposure to environmental EDCs and adrenocortical function, although the importance of the role of glucocorticoids in immune suppression is known (section 5.4.1.2). The role of altered hormonal homeostasis in early life in the generation of disease in later adult life is a relatively new field of research (Marmot and Wadsworth, 1997) and is clearly of potential relevance to the endocrine disruptors debate. For example, the possible role of elevated glucocorticoid levels in fetal life in later development of diabetes has already been mentioned (Chapter 3, section 3.2.3). These areas undoubtedly need further investigation.