Teratology Society Public Affairs Committee Position Paper: Developmental Toxicity of Endocrine Disruptors to Humans[†]

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In recent years there has been a growing concern that exposure to chemicals in the environment poses a serious threat to human reproduction and development via disrupting effects on endocrine function (Colborn and Clement, '92; Colborn et al., '93; Sharpe and Skakkebaek, '93, Toppari, et al., '96). An "endocrine disruptor" has been broadly defined as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock et al., '96). Due to the critical role of hormones in directing differentiation in many tissues, the developing organism is particularly vulnerable to fluctuations in the timing or intensity of exposure to chemicals with hormonal (or anti-hormonal) activity. Concerns have reached such a level that legislative requirements for identifying chemicals with endocrine disrupting potential were included in the Food Quality Protection Act and the Safe Drinking Water Act, which were passed by the U.S. Congress in 1996. The regulatory requirements emerging from these Acts will shortly begin to generate a considerable amount of information regarding the potential of a wide variety of chemicals to interact with various components of the endocrine system (US EPA, '98). More recently, the European Parliament voted to recommend the use of the precautionary principle in dealing with the regulation of chemicals with endocrine-disrupting actions (European Parliament, '98).

This position paper explores the relevant evidence from wildlife and laboratory animal studies on the effects of endocrine-disrupting chemicals and provides a critical examination of the evidence for the occurrence and magnitude of human health effects, particularly those mediated during development by the effects of environmental estrogens, anti-estrogens, androgens, anti-androgens, and chemicals affecting thyroid gland function. Endpoints relevant to the assessment of possible risks from exposure to endocrine disruptors during the prenatal or neonatal period include the development of structure and function of the male and female reproductive tracts, neurological development, onset of puberty, gender identity, sexual behavior, oocyte number, gonadotrophic and sex hormone levels, semen quality, fertility, pregnancy outcome, sex ratios, neoplastic and non-neoplastic changes in the gonads and secondary sex organs and tissues, and onset of menopause. The most prominent endpoints of potential developmental origin include congenital abnormalities (e.g., hypospadias and cryptorchidism), testicular cancer, reduced semen quality, and neurobehavioral alterations. Information gaps and research needs to address controversial issues and to better assess the magnitude of exposure and risk of adverse effects are also identified.

SUPPORTING EVIDENCE OF THE DEVELOPMENTAL TOXICITY OF ENDOCRINE DISRUPTORS

Wildlife studies

Considerable evidence for adverse effects of endocrine-disrupting chemicals has come from studies of wildlife

populations exposed to environmental contaminants. Effects have been observed at all levels of biological organization, from elevated biomarkers of exposure to behavioral disturbances, overt malformations, and, ultimately, population declines. Such effects have been observed in a variety of phyla and classes, including invertebrates, fish, reptiles, birds, and mammals. Due to the focus on human health, only selected examples are briefly mentioned here; readers are directed to recent reviews for more information (e.g., Umweltbundes Amt, '95; US EPA, '97; Daston et al., '97; Ankley et al., '98; Olsson et al., '98).

In the case of imposex (formation of a penis and vas deferens) in female molluscs, exposure to the antifouling agent tributyltin (a former constituent of marine paints) has been demonstrated to alter the activity of enzymes involved in the synthesis of estradiol (Ellis and Pattisina, '90). In populations of fish from several geographical regions, vitellogenin (an estrogen-inducible egg yolk protein) has been detected in the serum of male fish. The source of the estrogenic exposure appears to be both anthropogenic (ethynyl estradiol from birth control pills as well as natural estrogens that are excreted by women and eventually released into rivers from sewage treatment plants), and industrial (surfactants including alkyl phenol ethoxylates in effluents) and other as yet unidentified chemicals depending on the location (Mattheissen et al., '98). Reproductive failure, genital abnormalities, and decreased survival have been observed in alligators in Lake Apopka, Florida. These effects have been associated with a large spill of pesticides, including dicofol and DDT (Guillette et al., '94). The biological outcomes may be related to the anti-androgenic effects of p,p′-DDE, a persistent metabolite of DDT. Injections of 3–10 µg/kg egg mass of p,p′-DDE induced female phenotypes at otherwise male producing temperatures in the alligator (Matters et al., '98). Observations of early life stage mortality in Lake Trout from Lake Ontario (Ankley and Giesy, '98) and malformations such as cross-beaks in Lake Michigan cormorants (Giesy et al., '94) have been clearly associated with the levels of chemicals that act as Ah receptor agonists, but the linkage to endocrine disruption has not been specifically established. As expected due to the sensitivity of the developing organism to various endocrine disrupting modes-of-action, the effects in wildlife are generally most evident on the developing organism or on reproductive success. Similarities and differences between endocrine-disrupting effects in wildlife and humans have been reviewed from a risk-assessment perspective (Kavlock and Ankley, '96).

Laboratory animal studies

A variety of chemicals with various modes of interaction with the endocrine system have been tested in laboratory animal models and a wealth of information regarding adverse effects on development have been noted. Representative examples of types of chemicals,

principal modes of action, effective dose ranges and critical periods (to the extent currently understood), and characteristic phenotypes are summarized in Table 1. Pertinent aspects of these findings are discussed below.

Chemicals with estrogenic activity are a well-described class of developmental toxicants based on standard criteria of causing specific malformations during critical developmental periods of relatively short duration (Schardein, '93). The malformations increase both in severity and incidence with dose, although as noted below non-monotonic dose-response relationships have been noted for a few endpoints. Estrogens induce pleiotropic effects, acting on many types of cells with estrogen receptors, and can display cell and organ-specific agonist and antagonist actions. The pattern of outcomes is generally similar across different estrogens, although not all possible outcomes have been described for each. Diethylstilbestrol (DES) provides one of the most well-characterized examples of the effects of an estrogen on development. Manifestations of DES exposure include malformations and adverse functional alterations of the male and female reproductive tract and brain. In the CD-1 mouse, effective exposures are in the range of 0.01–100 µg/kg on GD 9–16 (Newbold, '95). At the higher end of the exposure range (10–100 µg/kg), total sterility of female offspring is noted, due in part to structural abnormalities of the oviduct, uterus, cervix, and vagina and to depletion and abnormalities of ovarian follicles. In adulthood, offspring show excessive vaginal keratinization, hypospadias, and epidermoid tumors of the vagina. Vaginal adenocarcinoma is seen at doses as low as 2.5 µg/kg. Benign uterine tumors (leiomyomas) are seen as low as 0.1 µg/kg. In male offspring, sterility is observed at high doses, the result of retained rete testes and Mullerian duct remnants, abnormal sperm morphology and motility, lesions in the reproductive tract (including cryptorchidism and rete testis adenocarcinoma), abnormal reproductive tract secretions, and inflammation (Newbold, '95).

Estrogenic and anti-estrogenic effects on development have also been reported for a number of synthetic and natural chemicals, including endogenous estrogens such as estradiol (Greene et al., '40; Biegel et al., '98, Cook et al., '98), estrogenic drugs such as ethynyl estradiol, anti-estrogenic drugs such as tamoxifen and clomiphene citrate (Branham et al., '88), and pesticides and industrial chemicals such as methoxychlor (Gray et al., '89), o,p′-DDT (Heinrichs et al., '71), kepone (Gellert, '79; Guzelian, '82), dioxins (Murray et al., '79, Mably et al., '92, Gray et al., '95), bisphenol A (Nagel et al., '97), and phytoestrogens such as genistein and coumestrol (Medlock et al., '95). Female offspring are generally more sensitive than males and altered pubertal development, reduced fertility, and reproductive tract anomalies are common findings. Tumorigenic responses have been observed only in studies with DES. A number of other chemicals have been shown either to

TABLE 1. Examples from laboratory animal models of the effects of exposure to endocrine disruptors during development

Chemical (use)	Mode of action	Exposure period	Dose range	Prominent effects	References
Estradiol (hormone)	ER agonist	Rat, one generation	0.05–50 ppm in diet (0.003–4.12 mg/kg/d)	Infertility at 10 and 50 ppm; at 2.5 ppm: delay in male preputial separation and acceleration in vaginal opening; hyperplasia of the mammary gland; decreased testes size; uterine epithelial hypertrophy and squamous metaplasia; decrease in epididymal sperm number but no change in Sertoli cell number.	Biegel et al. ('98); Cook et al. ('98)
DES (drug)	ER agonist	Mouse, GD 9–16	0.01–100 µg/kg	At 100 µg/kg, infertility in males and females, adenocarcinoma of vagina and rete testis adenocarcinoma; subfertility in females down to 2.5 µg/kg.	Newbold ('95)
Methoxychlor (insecticide)	ER agonist; AR antagonist	Rat, one generation	25–200 mg/kg/d	Accelerated puberty and reduced fertility and fecundity in female offspring at 50 mg/kg; delayed puberty (100 mg/kg), reduced growth (25 mg/kg), and reduced caudal sperm count (50 mg/kg) in male offspring.	Gray et al. ('89)
Flutamide (drug)	AR antagonist	Rat, GD 12–21	18–300 mg/kg/d	Adult males treated in utero had significantly smaller seminal vesicle and prostate weights at 18 mg/kg/d; feminized external genitalia, absent prostates and prominent nipple retention at 24 mg/kg/d and above; Wolffian duct differentiation effects (absent vas deferens, remnants of epididymides) at 100 mg/kg/d.	Imperato-McGinley et al. ('92)
Vinclozolin (fungicide)	AR antagonist	Rat, GD 14–PND 3	100 or 200 mg/kg/d	In males at both dose levels, reduced anogenital distance at birth, retained nipples, cleft phallus and hypospadias, suprainguinal testes, vaginal pouches, epididymal granulomas, small to absent sex accessory glands. Only effect in female was reduced anogenital distance in neonatal period.	Gray et al. ('94)
p,p′-DDE (insecticide metabolite)	AR antagonist	Rat, GD 14–18	10 or 100 mg/kg/d	Highest dose reduced male anogenital distance, increased retention of nipples, and altered expression of androgen receptor.	Kelce et al. ('95), You et al. ('98)
Finasteride (drug)	5α-reductase inhibitor	Rat, GD 6–20	0.0003–100 mg/kg/d	Reduced anogenital distance (4.2%) in male offspring at birth at 0.003 mg/kg/d (very shallow dose-response with persistent decrease with postnatal growth at higher doses); increased hypospadias and transient nipple retention in males at doses of 0.3 mg/kg/d and greater.	Clark et al. ('90)
Dibutylphthalate (plasticizer)	Unknown, but not AR ligand	Rat, GD 3–PND 20	250–750 mg/kg/d	Decreased anogenital distance at birth, absent prostate and seminal vesicles, small testes at >250 mg/kg; hypospadias and absent or undeveloped epididymis at 250 mg/kg and above.	Mylchreest et al. ('98)
Dioxin (environmental contaminant)	Ah agonist	Rat, GD 15	0.05–1 µg/kg	At 1 µg/kg, decreased anogenital distance in male and females; delayed puberty in females at 0.8 µg/kg and in males at 0.2 µg/kg; retained vaginal threads in females at 0.2 µg/kg; reduced ejaculated sperm count in males at 0.05 µg/kg.	Gray et al. ('97a,b)

bind to the estrogen receptor, induce estrogen-responsive genes in transcription assays, or to be uterotropic in animal tests (e.g., some phthalates and alkylphenols), but evidence for estrogen-receptor mediated developmental toxicity is generally lacking.

While most of the studies on estrogens have indicated traditional dose-response patterns of effects, with severity and incidence increasing with dose, vom Saal and workers (vom Saal et al., '97; Nagel et al., '97) have reported that unusual dose-response patterns for endocrine effects on some endpoints may occur. In those studies, a 50% increase above the control fetal serum estradiol concentration resulting from implantation of estradiol-containing Silastic™ capsules on days 13 to 19 of gestation in mice (a CF-1 strain maintained as an outbred, closed colony since 1979) caused a 30% increase in adult prostate weight, whereas higher maternal serum concentrations were associated with decreased adult prostate weight. A similar pattern was observed for DES given on days 11 to 17 of gestation, as increased adult prostate weights were seen between 0.02 and 20 ng/kg/d, whereas 200 ng/kg/d resulted in smaller prostates. Bisphenol A (2 and 20 µg/kg/d on gestation days 11 to 17) also increased adult prostate weight in these CF-1 mice, but higher doses were not given. These examples indicate an inverted U-shaped dose-response curve for this endpoint, and bring into question the design of hazard identification studies in the risk assessment of endocrine-mediated developmental responses. The significance and robustness of this observed phenomenon is controversial. For example, Cagen et al. ('99) found no change in prostate weight in approximately 90-day-old CF-1 mice whose mothers received between 0.2 and 200 µg/kg bisphenol A by deposition in the mouth on gestation days 11–17. This study was specifically designed to replicate the "critical" factors of the design used by vom Saal and coworkers, and employed larger sample sizes. Other studies, however, point to apparent low-dose linear responses for at least some estrogen receptor–mediated effects. For example, in the red-eared slider turtle, sex is determined by incubation temperature. At a female-temperature, sufficient levels of endogenous estrogens are synthesized to induce the female phenotype. Under these conditions, treatment with exogenous estradiol showed no threshold dose. A 20% increase above estimated endogenous estradiol concentration increased sex reversal by 14.4% (Sheehan et al., '99). Thus, for some endpoints, additivity of responses to endogenous and exogenous levels of hormone-receptor ligands may occur, further complicating the determination of potential risk to humans from low level exposures.

Attention has also become focused on chemicals that act via anti-androgenic mechanisms. Principal manifestations of developmental exposure to an anti-androgen are generally restricted to males, and include hypospadias, retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production. Ex-

amples of chemicals known to affect development via an anti-androgenic mechanism include pharmaceutical agents such as the androgen receptor antagonist flutamide (Imperato-McGinley et al., '92) and the 5a-reductase inhibitor finasteride (Clark et al., '90). Environmentally relevant compounds include the fungicide vinclozolin (Gray et al., '94), the DDT metabolite p,p'-DDE (Kelce et al., '95), and the herbicide linuron (Gray et al., '99), which are all androgen receptor antagonists. Recently, the phthalate esters dibutylphthalate and diethylhexylphthalate have been shown to induce an anti-androgen phenotype in developing rats, but the effect does not appear to be mediated by direct interaction with the androgen receptor (Mylchreest et al., '98, '99; Gray et al., '99). Other chemicals, such as the fungicide fenarimol (Gray et al., '91; Gray and Ostby, '98) and a metabolite of the insecticide methoxychlor (Maness et al., '98) also display anti-androgenic modes of action in in vitro or in vivo systems, but evidence of androgen-mediated developmental toxicity is lacking.

Hypothyroidism causes growth retardation, cognitive deficits, delayed eye opening, hyperactivity, and auditory defects. The most commonly used chemical to induce these outcomes is propylthiouracil. Polychlorinated biphenyls (PCBs) may act at several sites to lower thyroid hormone levels during development, and cause body weight and auditory deficits (Goldey et al., '95; Goldey and Crofton, '98). PCBs also cause learning deficits and alter locomotor activity patterns in rodents (Eriksson et al., '91; Schantz et al., '95) and monkeys (Bowman, '82; Schantz et al., '91). Some effects, such as deficits in spatial learning ability, closely resemble those seen following neonatal hypothyroidism (Porterfield, '94). However, at present there is no evidence causally linking the nervous system effects of PCBs to changes in the thyroid system. In fact, there does not seem to be a clear relationship between decreases in circulating thyroid hormone levels and spatial learning deficits in rats treated with specific PCB congeners (Schantz et al., '97). Hypothyroidism induced by neonatal treatment of rats with Arochlor 1254 also increases testis weight and sperm production by prolonging the period in which Sertoli cell proliferation is possible (Cooke et al., '96).

With the exception of hypothyroidism, most of the effects described above are thought to be due to alterations in sex steroid signaling pathways, either directly by altering receptor function, or by creating situations with inappropriate levels of endogenous receptor ligands. However, other mechanisms may be involved in causing adverse effects, including interference with steroidogenesis, hormone metabolism or elimination, as well as action via membrane receptors for peptide or steroid hormones, neurotransmitters, or orphan receptors. Other chemicals, such as DBCP and nitrofen, cause reproductive abnormalities following developmental exposure, but linkage to endocrine disruption is unclear (Cooper and Kavlock, '97).

EVIDENCE OF THE DEVELOPMENTAL TOXICITY OF ENDOCRINE DISRUPTORS TO HUMANS

Reports in humans that are or may be relevant to developmental toxicity from endocrine disruption are of two types: (1) Observations of adverse effects on reproductive system development and function following exposure to chemicals with known endocrine activities that are present in medicines, contaminated food, or the workplace. These have tended to involve relatively higher exposure to chemicals with known endocrine effects. (2) Epidemiologic evidence of increasing trends in reproductive and developmental adverse outcomes that have an endocrine basis. These reports have been linked speculatively with low-dose exposure to endocrine disruptors and have raised issues concerning long-term exposure to mixtures of chemicals with endocrine effects, some of which may bioaccumulate.

Observations in exposed cohorts

Besides a number of case reports involving exposure to hormonally active products used for therapeutic reasons (Schardein, '93), the most prominent evidence for adverse effects in humans is from the use of diethylstilbestrol (DES). A limited number of studies have focused on the effects of specific chemicals or chemical classes on lactation, breast cancer, and developmental neurotoxicity.

The DES experience. DES is a potent synthetic estrogen used for the prevention of spontaneous abortion in women during the 1950s and 1960s. Approval for its use was withdrawn in 1971 in the United States and in 1978 in Europe, following reports of vaginal clear-cell adenocarcinoma in teenage and young adult-age daughters of women who took DES during pregnancy (Herbst et al., '71). Other than vaginal clear cell carcinoma, no other increased cancer risk was found in a 16-year follow-up of three cohorts of women exposed to DES in utero (Hatch et al., '98). Unlike vaginal clear cell adenocarcinomas, which are relatively uncommon (around 1 in 1,000 exposed or less), congenital abnormalities of the reproductive tract are seen in up to 50% of DES-daughters, particularly tubal and uterine dysmorphologies, which contribute to a higher incidence of infertility and poor pregnancy outcomes such as spontaneous abortion, ectopic pregnancy, pre-term delivery, and low birth weight (Herbst and Bern, '81; Senekjian et al., '88). While prenatal or perinatal exposure to DES has caused masculinizing effects on female behavior in animal studies, reported effects on gender-related behavior in women exposed prenatally to DES are controversial (Newbold, '93; Hines, '92).

Sons of DES mothers also have an increased incidence of reproductive tract abnormalities, including cryptorchidism, microphallus, epididymal cysts, and reduced sperm count (Gill et al., '77, '79). However, fertility does not appear to be affected in these males (Wilcox et al., '95). No individual study of DES sons to date has shown any increase in testicular cancer, although a meta-analysis of published studies has suggested a marginally significant increase of approximately twofold in the incidence of testicular cancer among DES sons (Toppari et al., '96).

Lactational effects. The duration of lactation in a population of 229 Mexican women was reported to decrease from a median of 7.5 months to 3.0 months as the DDE content of milk increased from 0–2.5 ppm to >12.5 ppm (Gladen and Rogan, '95).

Breast cancer. It is not known whether prenatal exposure to estrogens plays any role in subsequent development of breast cancer in adult women but many of the risk factors for breast cancer are linked by the common feature of greater lifetime exposure to reproductive hormones. Studies showing an association between increased incidence of breast cancer and exposure to organochlorine compounds have also led to the hypothesis that xenobiotic compounds, such as PCBs, or DDT and its metabolites, acting as estrogens, could induce or promote breast cancer (Davis et al., '93). However, the largest study, which was prospective, measured PCBs and DDE in blood taken many years before, rather than around, the time of diagnosis of breast cancer, found no association (Krieger et al., '94). Recent extensive reviews also concluded that the existing evidence does not support a causal relationship between exposure to organochlorine compounds and either breast cancer, uterine cancer, or endometriosis, but neither does it provide sufficient grounds to reject such a hypothesis (Adami et al., '95; Ahlborg et al., '95). A more recent 17-year prospective study showed a twofold increased risk for breast cancer for dieldrin and confirmed the nonsignificant effects of PCBs and total DDT (Hoyer et al., '98).

Developmental neurotoxicity. Very few endocrine disrupting chemicals have been studied for nervous system effects. As discussed above, in utero exposure to PCBs causes learning deficits and alters locomotor activity patterns in rodents and non-human primates. Neurobehavioral changes and learning deficits have also been reported in children exposed to PCBs in utero or lactationally, either through their mothers' consumption of PCB-contaminated fish (Jacobson et al., '90; Jacobson and Jacobson, '96) or through exposure to background levels of PCBs in the United States (Rogan and Gladen, '91) and the Netherlands (Koopman-Esseboom et al., '96). In addition, there were two occurrences of high-level exposure to contaminated rice oil (in Japan in 1968 and in Taiwan in 1979) in which alterations in development of ectodermal tissues and delays in neurological development have been seen (Hsu et al., '85; Yu et al., '91; Guo et al., '94; Schecter et al., '94). In these cases, there was co-exposure to polychlorinated dibenzofurans as well as PCBs. While the pattern of effects seen with the lower levels of PCB exposure resembles that following neonatal hypothyroidism (Porterfield, '94) and several investigators (e.g., Brouwer et al., '98; Porterfield and Hendry, '98; Maclusky et al., '98) have speculated that the neurological

deficits are mediated via the thyroid system, there is, at present, no evidence causally linking them.

Secular trends

The second line of evidence suggestive of effects in the human population is from historical data indicating that a number of adverse birth outcomes and health effects that have a potential hormone-mediated origin and a critical developmental component have been on the rise through time. In all these cases, the evidence remains circumstantial due to the lack of exposure information and/or other identified risk factors.

Cryptorchidism. The incidence of cryptorchidism at age 3 months was reported as 0.97% in 3,612 male infants examined in London in the mid-1950s (Scorer, '64), compared with 1.78% in 7,400 male infants examined in Oxford between 1984 and 1988 using the same methods (Ansell et al., '92). A study of 6,935 male infants in New York, between 1987 and 1990, also using the same methods as Scorer ('64), reported an incidence of 1.0% at 3 months of age (Berkowitz et al., '93). Estimates of the incidence of cryptorchidism recorded at birth and up to adulthood vary from 0.03–13.4% (Toppari et al., '96). Cryptorchidism in the U.S. human population presently occurs at about a 0.4% incidence in neonates (Paulozzi, '99).

Hypospadias. The observed prevalence of hypospadias at birth in different geographic locations varies from 0.37–41 per 10,000 infants (Toppari et al., '96). The wide variations are probably attributable to known differences in diagnostic and reporting criteria. Increases over time have been reported in England and Wales, Hungary, Sweden, Norway, and Denmark. However, in Finland, Spain, New Zealand, Australia, and Czechoslovakia, the prevalence of hypospadias has not changed significantly over time (Toppari et al., '96). In England and Wales, the incidence now appears to be falling, following a steady increase between 1965 and 1983 (Medical Research Council, '95). The reasons for this are not known. Analyses of two American surveillance systems (the Metropolitan Atlanta Congenital Defects Program and the nationwide Birth Defects Monitoring Program (BDMP)) have indicated that the rates of hypospadias have nearly doubled since the 1970s and reached 39.7 per 10,000 births in 1993 in the BDMP. The authors felt it was unlikely that this increase was due to diagnostic changes or higher reporting rates (Paulozzi et al., '97).

Semen quality. Changes in semen quality have been examined over time and in different geographical areas. A meta-analysis of 61 studies published between 1938 and 1990, comprising results from 14,947 normal men of either proven fertility (39 studies) or unknown fertility (22 studies), suggested a decline in sperm concentration and semen volume over the last 50 years (Carlsen et al., '92). Twenty-eight of the studies were from the United States, 7 from Scandinavia, 11 from other European countries, and the remaining 15 from India, South America, Africa, Asia, and Australia. Using a weighted linear regression analysis, these authors concluded that sperm counts showed a significant decrease from a mean of 113×10^6/ml in 1940 to 66×10^6/ml in 1990, while semen volume fell from 3.40 ml to 2.75 ml over this period.

Subsequent commentary and replies from the original authors have discussed inevitable shortcomings of such a meta-analysis (Brake and Krause, '92; Bromwich et al., '94; Keiding et al., '94a,b; Skakkebaek and Keiding, '94; Farrow, '94; Olsen et al., '95). Potential selection bias within individual studies and from the selection of studies for the meta-analysis, variability in semen collection and analysis, especially with respect to period of abstinence, and the appropriateness of the linear regression model applied given the nature of the data and the paucity of data before 1970 have been debated. Re-analyses of the studies published between 1970 and 1990, which accounted for 88% of the total number of subjects in the meta-analysis, led to the conclusion that the data are compatible with a decline, no change, or an improvement in semen quality (Brake and Krause, '92; Bromwich et al., '94; Olsen et al., '95). The most rigorous re-analysis that used multiple regression analysis to control for abstinence time, age, percent proven fertility, specimen collection method, study goal, location, and group size concluded that a decline in sperm density was seen in the United States and Europe, but not in non-Western countries between 1938 and 1988 (Swann et al., '97).

Some recent reports from individual centers in Belgium, France, and Scotland have reported deteriorating semen quality over time (van Waeleghem et al., '94; Auger et al., '95; de Mouzon et al., '96; Irvine et al., '96), while other centers in the United States, Finland, and France have reported no change (Wittmaack and Shapiro, '92; Suominen and Vierula, '93; Vierula et al., '96; Bujan et al., '96; Paulsen et al., '96). Questions have again been raised concerning selection bias, but these studies have focused attention on alternative hypotheses, i.e., that there may be marked geographical variations in semen quality (Fisch and Goluboff, '96) but no decline over time, or a decline in semen quality over time in some areas but not others, rather than a general and widespread decline over time worldwide. It is notable that similar debates over differing findings on semen quality were taking place in the 1970s and 1980s, well before the emergence of the endocrine disruptor issue (Nelson and Bunge, '74; MacLeod and Wang, '79; James, '80; Leto and Frensilli, '81; Bostofte et al., '83; Bendvold, '89).

Only one study has addressed the possible consequences of geographical differences in sperm quality in relation to fertility. In an analysis designed to test the hypothesis that a higher sperm concentration in Finnish men compared with British men would be reflected in fertility, as measured by time to pregnancy, a comparison of two pairs of antenatal and cross-sectional studies conducted between 1983 and 1991 showed significantly

higher fertility in the Finnish men compared with the British men (Joffe, '96).

Sex ratios.   Analysis of the sex ratios in live births in Canada between 1930 and 1990 indicates that the proportion of male offspring decreased significantly after 1970, resulting in a cumulative loss by 1990 of 2.2 males per 1,000 live births; a similar, but lesser trend was evident in data from the United States (Allan et al., '97). Another analysis of live births in the United States between 1969 and 1995 showed a significant decline in male births among whites, but an increase among blacks. The authors discounted environmental exposures for the trends (Marcus et al., '98). Using data from 29 countries for birth ratios between 1950 and 1990, the proportion of males declined in 16 (some northern and southern European countries, Greece, Portugal, and Mexico), increased in six, and remained stable in seven (Parazzini et al., '98). Davis et al. ('98), using birth records from national registries, found significant declines in the proportion of males born since 1950 in Denmark and the Netherlands, and since 1970 in the United States, Canada, Sweden, Germany, Norway, and Finland. No risk factors were identified. A large increase in the ratio of female to male births has been noted in the Seveso area following the release of dioxins (Mocarelli et al., '96). James ('98) has postulated that gonadotropin levels around the time of ovulation can influence the relative number of female and male conceptuses.

Testicular cancer.   Trends in testicular cancer rates in countries as far apart as Europe, North America, and Australasia provide evidence of a marked increase of around 60–70% in many (but not all) countries over the last four decades (Toppari et al., '96). The increase is particularly evident among Caucasian men aged 20–35 years, the age of peak incidence for testicular cancer. However, some countries and racial groups have low baseline rates and do not show an increasing trend, including Finland which is geographically close to Denmark, the country with the highest known rate of testicular cancer (Toppari et al., '96). While there are racial and geographical differences in baseline rates, the increasing trend in many countries is clear, with incidence rates in some Nordic countries showing increases of 2–4% per year in men under 50 years of age. Importantly, cryptorchidism (see above) is a known risk factor for testicular cancer.

Other cancers of possible developmental origin.   Several other hormonally regulated cancers, including ovarian, uterine, and prostate, have been increasing over time in certain geographical areas (Devesa et al., '87; Ewertz et al., '89; Kavlock et al., '96). Some of the increase may be attributed to improved ascertainment. The contributions of developmental exposures to these outcomes have not been established.

CONCLUSIONS
DES as a model

The literature on DES provides clear evidence of reproductive tract malformations and cancers in female offspring in both humans and animal models. No individual study of DES sons has shown an increase in testicular cancer, but a meta-analysis found a significant increase, and high doses cause testicular cancer in mice exposed during development. Although structural abnormalities of the reproductive tract are seen in sons of women who took DES during pregnancy, fertility was generally found not to be affected. In using DES as a model for other endocrine-disrupting chemicals, however, it must be remembered that the effects occurred in populations receiving intended pharmaceutical-level exposure to a potent estrogen. Conversely, initiation of DES treatment was generally after the major period of sexual differentiation due to its use for threatened spontaneous abortion, and thus there are incumbent limitations in drawing conclusions about exposures at other times of gestation. The question of whether environmental chemicals, many of which have considerably lower binding affinity for the estrogen receptor (recognizing that low-affinity need not equate to low potency due to other factors such as metabolic persistence and serum binding protein affinities), may influence human reproductive health remains open. The carcinogenic potential of other chemicals with endocrine activity following prenatal or perinatal exposure has not been well studied.

Structural abnormalities of the reproductive tract

Hypospadias and cryptorchidism are two physical abnormalities observed in male offspring in experimental animal studies with chemicals that perturb androgen action. Several countries have noted an increase in hypospadias over time while a similar number of countries observed no change. Two countries that reported an increase now appear to be noting a steady decrease in incidence. The incidence of the female equivalent to hypospadias remains unstudied in humans, although such effects have been observed in DES mice. Lack of longitudinal studies and consistent diagnostic criteria compromises the utility of current human data to establish trends. Without such basic information, attempting to study associations between such effects and chemical exposures is not possible.

Semen quality

Experiments with both estrogenic and anti-androgenic chemicals have noted decrements in semen production in offspring of rodents treated during perinatal development. However, there are no exposure-effect studies on human semen quality, and the meta-analyses of the human semen quality studies published between 1970 and 1990 concluded that the data are compatible with a decline, no change, or an improvement in semen quality. It is difficult, if not impossible, to draw firm conclusions from the retrospective analyses of sperm count data collected for other purposes, but there are indications of marked variations in semen quality across various geographic locales. A clearer

understanding of specific chemicals to which populations are exposed and the timing, duration, and dose associated with their patterns of use are needed before firm scientific judgments can be formed.

Sex ratio

While the proportion of male offspring appears to have declined in a number of developed countries, including Canada, the United States, and several northern European countries, no known risk factors have been identified. In one population receiving high-level exposure to dioxin, an increase in the ratio of female to male births was observed.

Testicular cancer

Testicular cancer rates in North America and a number of other countries have shown a marked increase over the past four decades. It is noted that some countries and racial groups have low baseline rates and do not show an increasing trend. Despite the linkage of DES exposure to testicular cancers in laboratory animal models and possibly in humans, there are no studies that have examined the link between temporal trends in the general population and exposure to environmental chemicals.

Other cancers

The potential carcinogenicity of a number of organochlorine chemicals has been studied using traditional bioassays that commence exposure at about six weeks of age in rodents. A number have been shown to increase the incidence of hepatocellular tumors, usually in males. For several chemicals, such as dioxin and differing PCB mixtures, a significant reduction in mammary tumors was observed in females in these 2-year studies. Recent extensive reviews concluded that existing human data does not support a causal relationship between exposure to organochlorine compounds and breast cancer for PCBs or DDT and it metabolites, but do suggest a relationship with another organochlorine, dieldrin.

Neurobehavioral effects

Despite the fact that hormones play a central role in central nervous system development and function, very few chemicals classified as endocrine disruptors have been evaluated for neurobehavioral effects. An exception is PCBs. Perinatal exposure to PCBs has been associated with learning deficits in rodents, monkeys, and humans. A number of investigators have speculated that these effects are mediated through the thyroid system. However, at this time, there is no mechanistic evidence linking the cognitive changes to alterations in endocrine function.

SUMMARY AND RECOMMENDATIONS

It is the position of the Teratology Society that the data presently available do not support a consensus view that chemicals present in the environment are contributing to observed increases in human developmental disorders that are potentially endocrine related.

This conclusion is based on the facts that background historical data are lacking on many of these endpoints, that knowledge of geographical and time trends is very limited, and that exposure assessments have been virtually absent from the published literature. Nevertheless, there is considerable evidence that such effects have been found in some wildlife populations and in numerous, well-designed laboratory toxicology studies. Clearly chemicals with endocrine-disrupting actions have profound and predictable effects on the development of animals exposed during the perinatal period. Some of these chemicals are persistent in the environment and are present in the human body and conceptus. In particular, low-level exposures to PCBs have been linked to delayed neurological development in several populations around the world (although the mode of action remains to be established). Additionally, in a number of countries, phytoestrogens are a major source of exposure to non-pharmaceutical-, non-environmental-based chemicals that can interact with the endocrine system. While a number of beneficial attributes to the health of adults are associated with intake of phytoestrogens, virtually no studies of potential developmental effects in fetuses and children are available. On the assumption that responses of experimental animals to chemicals predict hazard to humans, extrapolation of the experimental findings raises serious and controversial questions and indicates that concern for similar effects in humans is warranted. Therefore, the Teratology Society is also of the view that endocrine disruption remains a viable hypothesis for some of the effects observed in humans and that additional research is needed to address the issue with greater certainty. Among these needs are:

- Case-control studies of congenital malformations of the reproductive tract, with emphasis on potential exposures to endocrine-disrupting chemicals.
- Determination of the nature and magnitude of observed geographical variations in reproductive tract malformations, semen quality and testicular cancer, and their relationship, if any, to exposure to endocrine-disrupting chemicals.
- Measurement of internal dose in populations likely to be exposed to endocrine-disrupting chemicals.
- Mechanistic studies to characterize the determinants of the dose-response patterns for exposures ranging from levels found in the environment to maximally-tolerated dosages.
- Comparison of risks from man-made vs. naturally occurring endocrine-active chemicals.
- Evaluation of potential interactive effects among endocrine-disrupting chemicals with dissimilar modes of action.

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