Water Qual. Res. J. Canada, 2001
Volume 36, No. 2, 253–271

Endocrine Disruption and Human Reproductive Effects: An Overview

WARREN G. FOSTER*

Center for Women's Health, Cedars-Sinai Medical Center, Los Angeles, California

Humans of all ages interact with their environment on a daily basis and are thus exposed to a variety of both man-made and naturally occurring chemicals through the air they breath, the water they drink and the food they eat. The potential for exposure to environmental contaminants to impact the function of the reproductive system and affect normal development of the reproductive tract has become an area of increasing concern at all levels of society. Environment Canada and Health Canada jointly organized a workshop to review the current state of knowledge on endocrine disrupting compounds (EDCs) and to establish a national science agenda on the scientific assessment of EDCs. This report summarizes the key scientific literature pertaining to the role of EDCs in a number of selected human reproductive/developmental outcomes. Change in the frequency of health outcome trends, epidemiological evidence of an association between the health outcome of concern and exposure to EDCs, and mechanistic evidence of receptor-mediated effects were the criteria used to evaluate the strength of the evidence. While it cannot be concluded that EDCs cause reproductive effects in the general Canadian population, the weight of evidence provides cause for continued concern.

Key words: **endocrine disruptors, reproduction, development, semen quality, endometriosis**

Introduction

The potential human reproductive and developmental effects of endocrine disrupting compounds (EDCs) has become an area of increasingly greater concern. These agents have been linked to a wide variety of adverse reproductive/developmental health outcomes in humans, including, but not limited to, the following: breast, ovarian and endometrial cancers, endometriosis, infertility, prolonged time-to-pregnancy, increased rates of spontaneous abortion, decreased birth sex ratios (number of males births/number of births for both sexes), increased prevalence of testicular cancer in young men, prostate cancer, decreased semen quality, increased frequency of testicular maldescent (cryptorchidism), increased prevalence of hypospadias (opening of the urethra along the shaft rather than the end of the penis), and precocious puberty. Meta-analyses of breast, ovarian and endometrial cancer have failed to find an association between these cancers and exposure to man-made chemicals (Adami et al. 1995; Houghton

* Corresponding author; warren.foster@cshs.org

and Ritter 1995). In contrast, a plausible argument has been put forward in the literature, suggesting that EDCs are contributing factors in the increased prevalence of testicular cancer of young men and developmental abnormalities of the male reproductive tract (Boyle et al. 1985; Carlsen et al. 1995; Jensen et al. 1995; Møller and Skakkebæck 1996; Toppari et al. 1996). However, the role of man-made chemicals and EDCs, in particular, in these adverse outcomes remains controversial.

There are a number of reports in the literature of trends in reproductive outcomes that suggest that environmental contaminants may be causing adverse effects on human reproduction and development of the male reproductive tract. For example, an increased prevalence has been reported for cryptorchidism and hypospadias in a number of countries (Källén et al. 1986), and most recently the United States (Paulozzi 1999). A decrease in the birth sex ratio (a decrease in the number of boys being born) has been reported in the United States (Allan et al. 1997; Scialli et al. 1997) as well as Canada (Allan et al. 1997) and elsewhere. Finally, there are also reports that body burdens of some contaminants are greater in women with endometriosis compared to a comparison group (Mayani et al. 1998). These reports, among others, taken together raise concerns regarding potential adverse effects of environmental contaminants on the reproductive tract and subsequent function. To test the weight of evidence supporting the hypothesis that EDCs are responsible for changes in human semen quality, developmental changes in the male reproductive tract, endometriosis and birth sex ratio, three criteria were used to evaluate the literature. Specifically, the strength of the data for (1) a change in the prevalence of the health outcome of interest; (2) the epidemiological evidence linking exposure and health effects; and (3) available mechanistic data indicating receptor-mediated disruption of an endocrine signalling pathway. While it is concluded that the accumulated evidence supports the plausibility of the hypothesis, the data do not support a conclusion that, at the levels of exposure reported for the general population in contemporary studies, human reproductive health has been adversely affected. The literature, however, is sufficiently strong to support continued concern and to warrant continued study of this problem. From this analysis a number of data gaps are identified and research priorities recommended.

Human Semen Quality and EDCs

The semen quality issue has possibly received the greatest attention of all adverse human reproductive outcomes in relation to EDC exposure in both the lay and scientific press. It is highly controversial and demonstrates clearly a number of the limitations of reports in this area of study. Attention was focused on a purported worldwide change in human semen quality with the appearance of the Carlsen and coworkers study (1992). The authors reviewed the global published semen quality literature and concluded that the concentration of human sperm declined by approximately 2% per year for the preceding 50 years. This study has been the focus of much debate in the literature and severe criticism due

to, among other issues, inadequate sample size in some of the included studies and improper statistical methodology (Olsen et al. 1995). Regardless, a subsequent reanalysis of the issue has supported the original findings (Swan et al. 1997) and thus attention continues to be focused on this problem.

A plethora of studies have appeared in the literature in recent years, some of which have demonstrated a decrease (Ginsburg et al. 1994; Auger et al. 1995; Adamopoulus et al. 1996; Irvine et al. 1996; Menchini-Fabris et al. 1996), no change (Bujan et al. 1992; Fisch et al. 1996; Paulsen et al. 1996; Handelsman 1997) or an increase (Vierula et al. 1996; Benshushan et al. 1997; Berling et al. 1997) in semen quality over time (Table 1). All of these studies, regardless of their outcome, suffer from sample selection bias since, in all cases, the subjects were selected from either fertility programs or men who were undergoing a vasectomy. Consequently, it is not possible to generalize from these studies to the whole population. Regardless, semen quality may serve as a useful biomarker of change in male fecundity (a man's potential to impregnate his partner) within a community.

Table 1. Annual percentage change in sperm concentration for various regions (published studies on changing semen quality)

City/Region/Country	% change/yr	Years	Reference
Athens	–0.94	1977–1993	Adamopoulos et al. 1996
North-eastern Spain	+0.04	1960–1996	Andolz et al. 1999
Paris	–2.1	1973–1992	Auger et al. 1995
Oslo	+0.5	1966–1986	Bendvold et al. 1989
Stockholm	–1.4	1956–1986	Bendvold et al. 1991
Copenhagen	–0.95	1952–1972	Bostofte et al. 1983
Toulouse	+0.3	1977–1992	Bujan et al. 1996
France	–1.75	1989–1994	de Mouzon et al. 1996
California	–0.98	1978–1994	Fisch et al. 1996
New York	+1.02	1972–1994	Fisch et al. 1996
Minnesota	+1.01	1971–1994	Fisch et al. 1996
London	+1.8% outside TWA	1978–1983	Ginsburg et al. 1994
	–4.8% inside TWA	1978–1983	Ginsburg et al. 1994
Edinburgh	–2.1%	1984–1995	Irvine et al. 1996
New York	+0.56	1966–1977	MacLeod and Wang 1979
Malmo	–2.37	1960–1980	Osser et al. 1984
Seattle	+0.13	1972–1993	Paulsen et al. 1996
Turku	+0.30	1967–1994	Vierula et al. 1996
Wisconsin	+2.62	1978–1987	Wittmaack and Shapiro 1992

Geographic differences in human semen quality are one of the more interesting and potentially important observations to develop from this entire issue. Regional differences within some countries such as Great Britain, France and Canada have been reported (Ginsburg et al. 1994; Fédération Française des CECOS 1997; YoungLai et al. 1998). Men living in the Thames River watershed were found to have lower sperm counts than were men living outside of the Thames River watershed and presumably obtaining their drinking water supply from a different and uncontaminated source (Ginsburg et al. 1994). Comparison of the results for two different cities in France demonstrated that semen quality has declined in Paris whereas there was a slight increase in semen quality in Toulouse (Fédération Française des CECOS 1997). In Canada, retrospective data collected from 11 different fertility clinics from across the country were compared (YoungLai et al. 1998). In this study, there were fertility clinics in some cities where semen quality had improved, others in which there were no differences, and finally clinics in which semen quality had declined. While the explanation for the reported differences (Ginsburg et al. 1994; Fédération Française des CECOS 1997; YoungLai et al. 1998) has not been demonstrated, these reports do suggest that regional differences may reflect locally important exposures to contaminants, dietary or lifestyle factors that should be investigated further in order to identify potential causative factors.

Since environmental contaminant residue levels were not determined in the studies cited above, it is not possible to conclude that where changes in semen quality have been observed, these findings are the consequence of exposure to chemical contaminants. Organochlorines and heavy metals have, however, been detected in human seminal plasma (Pines et al. 1987; Stachel et al. 1989; Chia et al. 1992; Noack-Fuller et al. 1993), indicating that chemical contaminants do reach the testis in concentrations that are detectable by current analytical chemistry methodologies. Moreover, impaired semen quality has been demonstrated for a number of different occupational groups in which exposure to chemical contaminants is inferred but not directly measured. An association with aromatic solvent exposure as determined by occupational questionnaires was associated with reduced semen quality in one study (Tielmans et al. 1999); however this study failed to control for confounding factors such as cigarette smoking and alcohol consumption. Semen quality was adversely affected in dry cleaners exposed to perchloroethylene in the workplace (Eskenazi et al. 1991). Occupational exposure to the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) has also been linked with decreased semen quality (Lerda and Rizzi 1991). No adverse health effects could be found in 67 men with occupational exposure to the anti-androgenic pesticide vinclozolin (Zober et al. 1995). Exposures varied in this study between 1 and 13 years and the levels of exposure exceeded the acceptable daily intake for approximately two-thirds of the subjects although the highest exposures were 10 times lower than the NOAEL determined from animal studies. In another study, semen quality was assessed in Danish farmers who used pesticides compared to reference group composed of organic farmers (Larsen et al. 1998). Although semen

quality was found to decrease in both groups, the magnitude of the change was similar and no statistical differences could be detected. In contrast, men exposed to the nematocide dibromochloropropane (DBCP) through their workplace experienced a severe disruption in fertility. DBCP-exposed men experienced a profound semen deficiency which in some cases was irreversible (Goldsmith 1997). In Canada, welders were also identified as an occupational at risk group for contaminant-induced effects on semen quality (Bigelow et al. 1998). Clearly, the literature reveals diminished semen quality in men with occupational or accidental exposure to man-made chemicals. However, effects of chemical exposures that result in the body burdens representative of the general population are unknown. It is conceivable that due to the inherent variability and the range of factors that affect human semen quality (illness, age, diet, medication, cigarette smoking, abstinence, etc.), it may be impossible to detect effects of background exposure levels. Furthermore, where occupational exposures have been associated with impaired semen quality, the mechanism of action of the associated contaminant have not been defined to the level that endocrine disruption can be concluded as the principle mechanistic pathway involved.

Potential mechanistic pathways for effects of EDCs on testicular function have been explored through animal experiments. Measures of sperm quality (number, motion, morphology, function) can be undertaken in animal studies and enable a direct comparison with humans. However, the process for the production of sperm is far more efficient in laboratory animal species, and thus small perturbations have a lesser chance to induce functional deficits. Therefore, it is reasonable to postulate that adverse effects in animals underestimate potential effects in human males due to the relative conservative production of gametes in humans versus rodents and non-human primate animal models. Regardless, from animal studies it is known that adult sperm production is dependent on the number of Sertoli cells formed during development and thus can be influenced by toxicants that affect Sertoli cell division either directly or indirectly. It has been demonstrated that Sertoli cells divide only during the prenatal and early postnatal periods of life (Orth 1982), and adult sperm production is dependent on the number of Sertoli cells produced during the perinatal period (Orth et al. 1988).

In the pubertal and adult male rat, the estrogenic contaminant methoxychlor was shown to antagonize the effects of androgens, which may indicate that methoxychlor or its metabolites are inhibiting both testosterone- and dihydrotestosterone (DHT)-induced gene expression and tissue growth and differentiation since some of the tissues lack functional estrogen receptors. Very high dose levels of methoxychlor can also alter fertility in the male rats by the inhibition of spermatogenesis whereas lower dose levels (25–200 mg/kg/d) reduce epididymal sperm reserves and seminal vesicle weight without affecting sperm production, testicular morphology or serum testosterone levels, indicating that different mechanisms may operate at different target tissue dose levels. Previous studies have also shown that gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced changes in

sperm concentration of exposed animals (Mably et al. 1992; Peterson et al. 1993; Bjerke and Peterson 1994). The actual mechanism of TCDD-induced suppression of sperm production has yet to be clarified.

Taken together, it is concluded that a worldwide decline in semen quality is unlikely but may be useful as a biomarker of effect as shown by potential regional differences, an observation that requires further investigation to identify potential causative agents. Evidence for at-risk occupational groups is provided and an effort to identify others is merited. Although effects have been documented in assorted occupational groups, the mechanism of action of the contaminants has not been determined. While animal studies are suggestive, further study of this issue is required to identify the key mechanistic pathways involved as well as the post-receptor signaling events. Such studies should be helpful, not only in characterizing the mechanistic pathways involved and identifying commonalties between humans and the model species, but should also help in elucidating some of the basic mechanistic physiology of spermatogenesis that has yet to be fully defined.

Developmental Effects of the Male Reproductive Tract

Concern that man-made chemicals may be altering reproductive development and function in the human population arises from reports of an increase in the frequency of developmental abnormalities in the male reproductive tract such as hypospadias (Källén et al. 1986) and cryptorchidism (Chilvers et al. 1984; Jackson 1988; John Radcliffe Hospital Cryptorchidism Study Group 1992). An increased prevalence of hypospadias was found in a descriptive epidemiological study (Källén et al. 1986), which utilized data from various malformation surveillance systems (Hungary, Sweden, Denmark, Italy, South America, Spain and Mexico). The incidence of hypospadias reported for South America and Mexico was the lowest whereas Hungary and Sweden had the highest rates for the years 1980 and 1981.

Analysis of the secular trends in the prevalence of crypthorchidism also indicates an increase over time. In one study, the prevalence of cryptorchidism was investigated by cohort analysis of patient discharge records for the years 1962–81 for England and Wales (Chilvers et al. 1984). This study 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, however, relied on the rate of orchiopexies (surgical repair of undescended testis, the procedure in which the testis are brought into the scrotal sac) carried out each year, and thus it is plausible that the entire increase in the described prevalence can be accounted for by a change in the criteria used to select patients for surgery. In contrast to this study, a prospective study was carried out by the John Radcliffe Hospital Cryptorchidism Study Group (1992) in which 7,441 boys were examined for cryptorchidism at birth and then again at three months of age during 1984–1988. The cryptorchidism rate at birth was found to have increased by 35.1%, and at three months of age by 92.7% over the rates reported by Scorer (1964).

A more exhaustive analysis of the trends of both of these defects of the male genital tract has recently been made (Paulozzi 1999). The birth prevalence rates for hypospadias and cryptorchidism were collected through the International Clearing House for Birth Monitoring System, a non-governmental organization related to WHO. The rates were systematically and continuously collected in 29 registries from 21 countries recording a total of 4 million births per year. A wide inter-country variation in rates of hypospadias and cryptorchidism around the world has been found. A factor of 3 or more could be observed between the highest rates (in the U.S. and Israel for hypospadias, U.S. 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 the comparison difficult. The secular evolution within various registries suggests an increase in hypospadias rates during the seventies and the eighties in U.S., Scandinavia and Japan. Nevertheless, no change was observed in Canada. No clear significant increase in cryptorchidism prevalence was observed. For both pathologies, a tendency towards a decline of rates was found after 1985.

In the only study of its kind that I am aware of, an increase in the incidence of cryptorchidism was reported for boys with higher levels of pesticide exposure as determined by place of residence (Garcia-Rodriguez et al. 1996). Unfortunately, this is a retrospective study without direct measurements of exposure, and thus potential exposure to factors other than pesticides cannot be excluded.

The cause of cryptochidism has yet to be determined. Risk factors associated with cryptorchidism include ethnicity, a family history of cryptorchidism, low birth weight, use of analgesics during pregnancy (Berkowitz and Lapinski 1996), birth order (Møller and Skakkebæk 1996), maternal obesity, cesarean delivery, pre-term birth and congenital malformations (Berkowitz et al. 1996). Lower circulating levels of testosterone have been demonstrated during gestation weeks 6–20 in boys with cryptorchidism (Key et al. 1996). It is therefore plausible that environmental contaminants which increase testosterone turnover decrease androgen synthesis, antagonize androgen action at the receptor level, act as an estrogen, or even possibly a combination of the proceeding could contribute to the increased prevalence of cryptorchidism.

The role of exogenous androgens was clearly demonstrated by *in utero* exposure to the 5α-reductase inhibitor Finasteride (blocks conversion of testosterone to DHT) and the anti-androgen Flutamide, which both exhibited significant disruptive effects on the development of the male reproductive tract (Imperato-McGinley et al. 1992). In animal experiments, cryptorchidism has been induced with a suspected estrogenic contaminant, mono-n-butyl phthalate, in rats (Imajima et al. 1997) and following treatment of pregnant sows with the anti-androgen flutamide (McMahon et al. 1995). The foregoing results from experimental animals suggest that either man-made chemicals with estrogenic activity or chemicals that block androgen action can induce cryptorchidism through receptor-mediated mechanisms. Mechanistic studies designed to examine

the effect of environmental contaminants on development of the male reproductive tract could be very helpful in identifying key target genes responsible for normal development and testicular descent. Finally, the impact of environmentally relevant concentrations of EDCs on development of the male reproductive tract requires further study.

Endometriosis

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 affecting approximately 14% of women of all reproductive ages (Vercillini et al. 1995). Although retrograde menstruation or bleeding into the peritoneal cavity during menstruation is widely accepted as a major contributing factor 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.

Chemical contaminants have recently been inculpated in the pathobiology of endometriosis as a result of a series of clinical-observational and animal studies. An association between endometriosis and exposure to PCBs (Gerhard and Runnebaum 1992) and dioxins (Koninckx et al. 1994) has been made. Koninckx and colleagues (1994) noted that the incidence and severity of endometriosis as well as the degree of dioxin pollution in Belgium is among the highest in the world. 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 women with tubal infertility (Mayani et al. 1997). Note that, although significantly more women with endometriosis (eight [18%] compared to one 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, while an OR of 7.6 was obtained, the 95% CI included unity (0.87–169.7). In another study, no association between organochlorine concentrations and endometriosis could be found in a case control study of 86 women with endometriosis and 70 controls matched for the indication of laparoscopy (Lebel et al. 1996). 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). Unfortunately these studies are all of a case control design and thus lack true controls (i.e., women without any exposure to man-made chemicals). In addition, these studies are all relatively small, and thus may not have the statistical power to detect differences if they were indeed present. It has previously been determined that a sample size of approximately 300 subjects with endometriosis and a similar number of 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). Hence, the human data at

present neither confirm nor refute the hypothesis that environmental contaminants play a role in the pathobiology of endometriosis.

The notion that environmental contaminants are involved in the pathophysiology of endometriosis gained momentum with the demonstration that the incidence and severity of spontaneous endometriosis was increased in rhesus monkeys following treatment with TCDD (Rier et al. 1993). In contrast, rhesus monkeys treated with PCBs failed to show any relationship between the incidence and/or severity of endometriosis and PCB dose (Arnold et al. 1996). Recently, the role of TCDD in the pathophysiology of endometriosis was studied in Cynomolgus monkeys with surgically induced endometriosis (Yang et al. 2000). The doses of TCDD employed in this study were selected to be equivalent to those used by Rier and colleagues (1993) and expanded to include one lower dose level. Interestingly, this study revealed a bimodal effect of TCDD on the maximum and minimum implant diameter. Maximal and minimum diameters of the lesions were significantly greater in the 25 ppt dose group compared to the controls and reduced in the 1 ppt group compared to controls. Survival of ectopic endometrial implants were also affected by TCDD treatment, with survival being significantly enhanced at the 5 and 25 ppt level compared to the controls. This study extends the findings of the Rier et al (1993) report and suggests that TCDD has multiple effects on the endometrium depending upon dose. However, the mode of action of TCDD on the non-human primate endometrium has yet to be explored.

Various rodent models have been introduced to examine the potential for EDCs to play a role in endometriosis. Ovariectomized mice with autotransplanted uterine strips were used to demonstrate that administration of 4-chlorodiphenyl ether, an estrogenic compound, could facilitate implant growth (Yang et al. 1993). Surgical induction of endometriosis in rodents has suggested that the disease can be enhanced by the administration of TCDD (Cummings et al. 1996; Johnson et al. 1997). The administration of very large doses of TCDD induced endometriosis in both the rat and the mouse with the mouse being seen as a more sensitive model for the growth of endometriotic implants (Cummings et al. 1996). In contrast, in ovariectomized and estrogen-replaced mice, TCDD treatment inhibited the growth of uterine implants (Yang and Foster 1997). These data suggest that ovarian factors other than estradiol contribute to the survival and growth of the implants. TCDD and polynuclear aromatic hydrocarbons interact with the aryl hydrocarbon receptor (AhR), a high affinity low capacity protein (Okey et al. 1979, 1980; Farrell et al. 1987), which is translocated into the nucleus to bind with dioxin response elements present in the cell DNA. There are no known endogenous ligands for this receptor and therefore it is considered an orphan receptor, although recent studies reveal that metabolites of tryptophan, namely, tryptamine and indole acetic acid, bind to and activate the AhR in culture (Heath-Pagliuso et al. 1998). AhR ligand binding affects a number of genes that are potentially important in the pathobiology of endometriosis. In particular, AhR ligand binding regulates epidermal growth factor (EGF), interleukin Iβ (IL-1β), and transforming growth factors (TGF-α and TGF-β) (Madhukar et al.

1984; Sutter et al. 1991; Gaido et al. 1992). Furthermore, in the rat, TCDD treatment inhibited 17β-estradiol-induced increase in uterine wet weight (Gallo et al. 1986), and in mice, the 17β-estradiol-induced increase in uterine EGF mRNA levels was suppressed. Contradictory reports have appeared in the literature concerning the effect of TCDD on uterine estrogen receptor levels, where TCDD induced suppression in rats has been reported by some (Romkes et al. 1987; Astroff et al. 1990), whereas others found no effect in mice (De Vito et al. 1994). Decreased expression of estrogen receptor mRNA in the ovaries and uteri of TCDD-treated mice has also been demonstrated (Tian et al. 1998). Consequently, it is concluded that the molecular mechanisms of TCDD effects on the uterus of rodents and the endometrium of primates has yet to be defined.

Rodents are considered by many to be poor models for endometriosis since they do not spontaneously develop endometriosis. Moreover, the physiology of implanted uterine fragments are unlikely to accurately reflect the physiology of human endometrial cells that spontaneously implant and result in endometriosis. Consequently, in order to clarify the role of EDCs in the pathophysiology of endometriosis, it will be necessary to develop better animal models of human endometriosis, in addition to determining the molecular mechanism of contaminant effects on endometrial physiology. Finally, there is a need for statistically robust prospective epidemiological studies that link exposure and endometriosis (Table 2).

The Sex Ratio

Declining sex ratios 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; Marcus et al. 1998), the Netherlands (van der 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 to occur in Latin American countries (Feitosa and Krieger 1992, 1993).

As in the case of semen quality, the effects are not homogeneous in the population throughout the entire country and thus environmental concerns have been raised. In the Allan study (1997), the lowest ratios were detected in the Atlantic provinces and Quebec. The authors also note that when the observation period is extended out, no overall change in the birth sex ratio was detectable. Variations in the birth-sex ratio distribution were also noted in the United States. Significant declines were also present in four of nine regions of the United States (East-North Central, West-North Central, South Atlantic and Pacific).

Regression analysis of secular trends in sex ratio of live births between 1969 and 1995 in the U.S. (Marcus et al. 1998) revealed a significant decline among whites for the 27 years under study (OR = 0.9935; 95% CI 0.9915–0.9915). 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).

Although there is poor understanding of all factors that lead to a change in the sex ratio, there is clear evidence that external influences are associated with such a change. In addition to the age of either the mother or the father as well as some medical conditions, parental hormone levels at the time of conception have been suggested to affect sex ratio (James 1986). Sas and Szollosi reported (1980) a higher sex ratio when men were administered testosterone during attempts to conceive. Although the sample size was small, the findings suggest that the relative estrogen:androgen ratio in men at the time of conception is important in determining the sex of embryos that implant and are subsequently delivered.

Occupational exposures have been significantly associated with changes to the sex ratio. Of interest, however, there were significant reductions in the numbers of males born to women whose partners were exposed to DBCP (Potashnik et al. 1984). This is despite the fact that the

Table 2. Proposed research priorities and study goals to address the issue of EDCs and adverse human reproductive/developmental effects

Study type	Study goals
Exposure assessment assessment	Expand to include exposure to endocrine active dietary factors, e.g., flavanoids. Cover all developmental periods, including *in utero* and pre-pubertal stages. Validate surrogate markers of exposure: food frequency questionnaires, occupational histories as well as umbilical cord blood as an indicator of *in utero* exposure.
Epidemiology	Prospective studies for semen quality. Identify and explore potential causative factors in regional effects. Link exposure with outcome of interest.
Hazard identification and risk characterization	Develop animal models for the outcomes of interest, e.g., endometriosis, sperm production or sex ratio. investigate potential interactions among contaminants Characterize the risk of exposure during each developmental stage. Explore the hazards associated with relevant exposure scenarios and dose levels. Explore mechanisms of action related to endocrine signaling. Investigate the role of dietary factors in adverse health outcomes, both as causative agents as well as potential confounders.

proportion of male births observed for the same men was 0.5 prior to exposure. The specific mechanism of action of how this chemical would have changed the sex ratio has not been advanced. Additionally, it has been suggested that exposure to organochlorine pesticides (de Cock et al. 1995) change the sex ratio. One Dutch study of the offspring born from 1978 to 1990 revealed a shift toward daughters when men had workplace exposure to pesticides. Vinclozolin, an anti-androgen, has also been reported to have the same effect (Dodds and Armson 1997).

Further evidence that environmental exposure may influence the male proportion of births comes from recent epidemiological reports of the Seveso, Italy, population who were exposed to TCDD as a consequence of an industrial accident. The male proportion of births in this population was significantly reduced among couples with the greatest exposures to TCDD between 1977 and 1984 (Mocarelli et al. 1996). It is interesting to note that since 1985 the male proportion of this population has returned to expected levels (Needham et al. 1997). The actual mechanism for this change in the proportion of male births has not been determined and although there is an assumption that endocrine modifications are involved, it has not been confirmed. As if to illustrate the frustrating lack of consistency of findings for exposures to the same contaminants and the difficulties associated with this area of study, no changes in the birth sex ratio were noted in the TCDD-contaminated region of Kazakhatan (Hooper et al. 1998). Divergent findings may be due to methodological issues, actual exposure levels, concomitant exposures, diet, general health status of the study subjects, interval from exposure to outcome measurement and sample size, to name but a few.

In animals, several lines of evidence suggest external factors might function through disruption of the endocrine system to alter the birth sex ratio. First, Vandenbergh and coworkers (1994) have shown that the intrauterine position of the mother affects the sex ratio of its offspring. The sex ratio of the first litter born to 2M females (female fetuses located between two males) was 58%, for 1M females was 51% male and for 0M females was 42%. These differences are maintained into the second litter. There were no changes in the number of pups born to mothers from different intrauterine positions. This information suggests that the hormonal milieu during gestation probably plays an important role in altering the subsequent birth sex ratio (Vandenbergh et al. 1994). Secondly, there is a recognized developmental difference in rates of male and female embryos. Male embryos in almost every species studied advance to the blastocyst stage first, and recent studies have shown there are differential rates of cell growth and division between XX and XY bearing mammalian embryos (Scott and Holson 1977; Pederson 1980; Tsunoda et al. 1985; Xu et al. 1992; Burgoyne 1993), and the differences are associated with the Y chromosome (Burgoyne 1993). This observation suggests that male embryos may be more sensitive to the actions of estrogens, anti-estrogens and anti-androgens. Alternatively, it can be proposed that male embryos may be more sensitive to the general toxicity of environmental contami-

nants. The effect of exogenous chemicals on birth sex ratio is an area that begs for greater attention at all levels of inquiry.

Summary and Conclusions

In summary, with the exception of occupational and accidental exposures, the available evidence does not support a conclusion that man-made chemicals have induced any changes in human reproduction or development of the male reproductive tract. Reports in the literature do, however, suggest changes in reproductive physiology and reproductive tract development are indeed plausible, but the mechanism(s) underlying and driving these changes remains undetermined. Demonstration that any of the adverse outcomes discussed in this report are or can be mediated by man-made chemicals acting via an endocrine pathway has yet to be established with certainty.

The potential for environmental contaminants to interact with the endocrine system and induce adverse effects in the human population is an issue that will remain important for some time to come. Research needs have been provided for each of the health issues discussed in this report and are summarized in Table 2. Briefly, it will be necessary for investigators that routinely measure human exposures to recruit people from the general population into their studies and, wherever possible, to forge collaborations with epidemiologists so that exposure and effects may ultimately be linked. Furthermore, it is essential that in addition to hazard identification, the mechanisms of action be determined utilizing the most appropriate animal models. In some cases, this will require the development of new animal models of human diseases such as endometriosis. It is expected that identification of the receptor-mediated pathways will contribute to evidence-based risk assessment decisions, but will also yield useful information for the elucidation of the basic physiology underlying normal reproductive function and development.

References

Adami H-O, Lipworth L, Titus-Ernstoff L, Hsieh C-C, Hanberg A, Ahlborg U, Baron J, Trichopoulos D. 1995. Organochlorine compounds and estrogen-related cancers in women. Cancer Causes and Control **6:**551–566.

Adamopoulos DA, Pappa A, Nicopoulou S, Andreou E, Karamertzanis M, Michopoulos J, Deglianni V, Simon M. 1996. Seminal volume and total sperm number trends in men attending subfertility clinics in the Greater Athens area during the period 1977–1993. Human. Reprod. **11:**1936–1941.

Allan BB, Brant R, Seidel JE, Jarrell JF. 1997. Declining sex ratios in Canada. Can. Med. Assoc. J. **156:**37–41.

Andolz P, Bielsa MA, Vila J. 1999. Evolution of semen quality in north-eastern Spain: a study in 22759 infertile men over a 36-year period. Hum. Reprod. **14:**731–735.

Arnold DL, Nera EA, Stapley R, Tolnai G, Claman P, Hayward S, Tryphonas H, Bryce F. 1996. Prevalence of endometriosis in rhesus (*Macaca mulatta*) mon-

keys ingesting PCB (Aroclor 1254): review and evaluation. Fund. Appl. Toxicol. **31:**42–55.

Astroff B, Rowlands C, Dickerson R, Safe S. 1990. 2,3,7,8-tetrachlorodibeno-*p*-dioxin inhibition of 17β-estradiol -induced increase in rat uterine EGF receptor binding activity and gene expression. Mol. Cell Endocrinol. **72:**247–252.

Auger J, Kunstmann JM, Czyglik F, Jouannet P. 1995. Decline in semen quality among fertile men in Paris during the past 20 years. New Engl. J. Med. **332:**281–285.

Bendvold E. 1989. Semen quality in Norwegian men over a 20-year period. Int. J. Fertil. **34:**401–406.

Bendvold E, Gottlieb C, Bygdeman M, Eneroth P. 1991. Depressed semen quality in Swedish men from barren couples: a study over three decades. Arch. Androl. **26:**189–194.

Benshushan A, Shoshani O, Paltiel O, Schenker JG, Lewin A. 1997. Is there a decrease in sperm parameters among healthy young men? A survey of sperm donations during 15 years. J. Assist. Reprod. Genet. **14:**347–353.

Berkowitz GS, Lapinski RH. 1996. Risk factors for cryptorchidism: a nested case-control study. Paediatr. Perinat. Epidemiol. **10:**39–51.

Berkowitz GS, Lapinski RH, Godbold JH, Dolgin SE, Holzman IR. 1996. Maternal and neonatal risk factors for cryptorchidism. Epidemiol. **6:**127–131.

Berling S, Wölner Hanssen P. 1997. No evidence of deteriorating semen quality among men in infertile relationships during the last decade: a study of males from Southern Sweden. Hum. Reprod. **12:**1002–1005.

Bigelow PL, Jarrell J, Young MR, Keefe TJ, Love EJ. 1998. Association of semen quality and occupational factors: comparison of case-control analysis and analysis of continuous variables. Fertil. Steril. **69:**11–18.

Bjerke D, Peterson R. 1994. Reproductive toxicity of 2,3,7,8,-Tetrachlorobenzo-p-dioxin in male rats: Different effects of in Utero versus lactational exposure. Toxicol. Appl. Pharmacol. **127:**241–249.

Bostofte E, Serup J, Rebbe H. 1983. Has the fertility of Danish men declined through the years in terms of semen quality? Int. J. Fertil. **26:**91–95.

Boyd JA, Clark GC, Walmer DK, Patterson DG, Needham LL, Lucier GW. 1995. Endometriosis and the environment: biomarkers of toxin exposure. Conference on Endometriosis 2000, May 15–17, 1995, Bethesda, National Institute of Health.

Boyle P, Kaye SB, Robertson AG. 1987. Changes in testicular cancer in Scotland. Euro. J. Cancer Clin. Oncol. **23:**827–830.

Bujan L, Mansat A, Pontonnier F, Mieusset R. 1996. Time series analysis of sperm concentration in fertile men in Toulouse, France, between 1977 and 1992. Br. Med. J. **312:**471–472.

Burgoyne PS. 1993. A Y-chromosomal effect on blastocyst cell number in mice. Development **117:**341–345.

Carlsen E, Giwercman A, Keiding N, SkakkebFk NE. 1992. Evidence for decreasing quality of semen during past 50 years. Br. Med. J. **305:**609–613.

Carlsen E, Giwercman A, Keiding N, SkakkebFk NE. 1995. Declining semen quality and increasing incidence of testicular cancer: Is there a common cause? Environ. Hlth. Perspect. **103(Suppl. 7):**137–139.

Chia SE, Ong CN, Lee ST, Tsakok FH. 1992. Blood concentrations of lead, cadmium, mercury, zinc, and copper and human semen parameters. Arch. Androl. **29:**177–183.

Chilvers C, Pike MC, Forman D, Fogelman K, Wadsworth MEJ. 1984. Apparent doubling of frequency of undescended testis in England and Wales in 1962–81. Lancet **2, 8398:**330–332.

Cummings AM, Metcalf JL, Birbaum L. 1996. Promotion of endometriosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats and mice: Time-dose dependence and species comparison. Toxicol. Appl. Pharmacol. **138:**131–139.

de Cock J, Heederik D, Tielemans E, te Velde E, van Kooij R. 1995. Offspring sex ratio as an indicator of reproductive hazards associated with pesticides. Occup. Environ. Med. **52:**429–430.

de Mouzon J, Thonneau P, Spira A, Multigner L. 1996. Declining sperm count. Semen quality has declined among men born in France since 1950. Brit. Med. J. **313(7048):**43–45.

De Vito MJ, Ma X, Babish JG, Menache M, Birnbaum LS. 1994. Dose-response relationships in mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: CYP1A1, CYP1A2, estrogen receptor, and protein tyrosine phosphorylation. Toxicol. Appl. Pharmacol. **124:**82–90.

Dodds L, Armson BA. 1997. Is Canada's sex ratio in decline? CMAJ. 156:46–48.

Eskenazi B, Wyrobeck AJ, Fenster L, Katz DF, Sadler M, Lee J, Hudes M, Rempel DM. 1991. A study of the effect of perchlorethylene exposure on semen quality in dry cleaning workers. Am. J. Ind. Med. **20:**575–591.

Farrell KL, Safe L, Safe S. 1987. Synthesis and Ah receptor binding properties of radiolabeled polychlorinated dibenzofuran congeners. Arch. Biochem. Biophys. **259:**185–195.

Fédération Française des CECOS. 1997. Evidence for regional differences of semen quality among fertile French men. Human Reprod. **12:**740–745.

Feitosa MF, Krieger H. 1992. Demography of the human sex ratio in some Latin American countries, 1967–1986. Hum. Biol. **64:**523–530.

Feitosa MF, Krieger H. 1993. Some factors affecting the secondary sex ratio in a Latin American sample. Hum. Biol. **65:**273–278.

Fisch H, Goluboff ET, Olson JH, Feldshuh J, Broder SJ, Barad DHB. 1996 Semen analysis in 1283 men from the United States over a 25-year period: no decline in quality. Fertil. Steril. **65:**1009–1014.

Gaido KW, Maness SC, Leonard LS, Greenlee WF. 1992. 2,3,7,8-Tetrachlorodibeno-*p*-dioxin-dependent regulation of transforming growth factor-α and β_2 in a human keratinocyte cell line involves both transcriptional and post-transcriptional control. J. Biol. Chem. **267:**24591–24595.

Gallo MA, Hesse EJ, MacDonald GJ, Umbreit TH. 1986. Interactive effects of estradiol and 2,3,7,8-tetrachlorodibeno-*p*-dioxin onhepatic cytochrome P-450 and mouse uterus. Toxicol. Lett. **32:**123–132.

Garcia-Rodriguez J, Garcia-Martin M, Nogueras-Ocana M. 1996 Exposure to pesticides and cryptorchidism: Georgraphical evidence of a possible association. Environ. Hlth. Perspect. **104:**1090–1095.

Gerhard I, Runnebaum B. 1992. Fertility disorders may result from heavy metal and pesticide contamination which limits effectiveness of hormone therapy. Zent bl Gynakol. **114:**593–602.

Ginsburg J, Okolo S, Prelevic G, Hardiman P. 1994. Residence in the London area and sperm density. Lancet **8891:**230.

Goldsmith JR. 1997. Dibromochloropropane: epidemiological findings and current questions. Ann. N.Y. Acad. Sci. **837:**300–306.

Gray Le Jr., Ostby J, Marshall R, Andrews J. 1993. Reproductive and thyroid effects of low-level polychlorinated biphenyl (Aroclor 1254) exposure. Fund. Appl. Toxicol. **20:**288–294.

Halme J, Hammond MG, Hulka JF. 1984. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol. **64:**151–154.

Handelsman DJ. 1997. Sperm output of healthy men in Australia: magnitude of bias due to self-selected volunteers. Hum. Reprod. **12:**2701–2705.

Heath-Pagliuso S, Rogers WJ, Tullis K, Seidel SD, Cenijn PH, Brouwer A, Denison MS. 1998. Activation of the Ah receptor by tryptophan and tryptophan metabolites. Biochem. **37:**11508–11515.

Hooper K, Petreas MX, Chuvakova T, Kazbekova G, Druz N, Seminova G et al. 1998. Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakstan: high levels of 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in agricultural villages of southern Kazakstan. Environ. Hlth. Perspect. **106:**797–806.

Houghton DL, Ritter L. 1995. Organochlorine residues and risk of breast cancer. J. Am. College Path. **14:**71–89.

Imajima T, Shono T, Zakaria O, Suita S. 1997. Prenatal phthalate causes cryptorchidism postnatally by inducing transabdominal ascent of the testis in fetal rats. J. Pediatr. Surg. **32:**18–21.

Imperato-McGinley J, Sanchez RS, Spencer JR, Yee B, Vaughan ED. 1992. Comparison of the effects of the 5 alpha-reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose-response studies. Endocrinol. **131:**1149–1156.

Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. 1996. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. Br. Med. J. **3121:**467–471.

James WH. 1986. Hormonal control of sex ratio. J. Theor. Biol. **118:**427–441.

Jackson MB and the John Radcliff Hospital Cryptorchidism Research Group. 1988. The epidemiology of cryptorchidism. Horm. Res. **30:**153–156.

Jensen TK, Toppari J, Keiding N, SkakkebFk NE. 1995. Do environmental estrogens contribute to the decline in male reproductive health? Clinical Chemistry **41:**1896–1901.

John Radcliffe Hospital Cryptorchidism Study Group. 1992. Cryptorchidism: a prospective study of 7500 consecutive male births, 1984–8. Arch. Dis. Child. **67:**892–899.

Johnson KL, Cummings AM, Birnbaum LS. 1997. Promotion of endometriosis in mice by polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls. Environ. Hlth. Perspect. **105:**750–755.

Källén B, Bertollini R, Castilla. 1986. A joint international study on the epidemiology of hypospadias. Acta. Paediatr. Scand. [Suppl] **324:**5–52.

Key TJ, Bull D, Ansell P, Brett AR; Clark GM; Moore JW; Chilvers CE; Pike MC. 1996. A case-control study of cryptorchidism and maternal hormone concentrations in early pregnancy. Br. J. Cancer **73:**698–701.

Koninckx PR, Braet P, Kennedy SH, Barlow DH. 1994. Dioxin pollution and endometriosis in Belgium. Hum. Reprod. **9:**1001–1002.

Larsen SB, Giwercman A, Spano M, Bonde JP, the ASCLEPIOS Study Group. 1998. A longitudinal study of semen quality in pesticide spraying Danish farmers. Reprod. Toxicol. **12:**581–589.

Lebel G, Dodin S, Ayotte P, Marcoux S, Ferron LA, Dewailly E. 1998. Organochlorine exposure and the risk of endometriosis. Fertil. Steril. **69:**221–228.

Lerda D, Rizzi R. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mut. Res. **262:**47–50.

Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE. 1992. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 3. Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharm. **114:**118–126.

MacLeod J, Wang Y. 1979. Male fertility potential in terms of semen quality: a review of the past, a study of the present. Fertil. Steril. **31:**103–116.

Madhukar BV, Brewster DW, Matsumura F. 1984. Effects of in vivo-administration 2,3,7,8-tetrachlorodibeno-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. Proc. Natl. Acad. Sci. USA **81:**7407–7411.

Marcus M, Kiely J, Xu F, McGeehin M, Jackson R, Sinks T. 1998. Changing sex ratio in the United States, 1969–1995. Fertil. Steril. **70:**270–273.

Mayani A, Barel S, Soback S, Almagor M. 1997. Dioxin concentration in women with endometriosis. Human Reprod.**12:**373–375.

McMahon DR, Kramer SA, Husmann DA. 1995 Antiandrogen induced cryptorchidism in the pig is associated with failed gubernacular regression and epididymal malformations. J. Urol. **154:**553–557.

Menchini-Fabris F, Rossi P, Palego P, Simi S, Turchi P. 1996. Declining sperm counts in Italy during the past 20 years. Andrologia **28:**304.

Mocarelli P, Brambilla P, Gerthoux PM, Patterson Jr. DG, Needham LL. 1996. Change in sex ratio with exposure to dioxin. Lancet **348:**409.

Møller H. 1996. Change in male:female ratio among newborn infants in Denmark. Lancet **348:**828–29.

Møller H. 1998. Trends in sex-ratio, testicular cancer and male reproductive hazards: are they connected? APMIS **106:**232–38.

Møller H, Skakkebaek NE. 1996. Risks of testicular cancer and cryptorchidism in relation to socioeconomic status and related factors: Case-control studies in Denmark. Int. J. Cancer. **66:**287–293.

Needham LL, Gerthoux PM, Patterson DG, Jr., Brambilla P, Turner WE, Beretta C et al. 1997. Serum dioxin levels in Seveso, Italy, population in 1976. Terat. Carcin. Mutagen. **17:**225–40.

Noack-Fuller G, DeBeer C, Seibert H. 1993. Cadmium, lead, selenium, and zinc in semen of occupational unexposed men. Andrologia **25:**7–12.

Okey AB, Bondy GP, Mason ME, Kahl GS, Eisen HJ, Guenthner TM, Nebert DW. 1979. Regulatory gene product of the Ah locus: characterization of the cytosolic inducer-receptor complex an evidence for its nuclear translocation. J. Biol. Chem. **254:**11636–11648.

Olsen GW, Bodner KM, Ramlow JM, Ross CE, Lipshultz LI. 1995. Have sperm counts been reduced 50 percent in 50 years? A statistical model revisited. Fert. Steril. **63:**887–893.

Orth JM. 1982. Proliferation of Sertoli cells in fetal and postnatal rats: A quantitative autoradiographic study. Anat. Record. **203:**485–92.

Orth JM, Gunsalus GL, Lamperti AA. 1988. Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinol. **122:**787–794.

Osser S, Liedholm P, Ranstam J. 1984. Depressed semen quality: a study over two decades. Arch. Androl. **12:**113–116.

Paulozzi LJ. 1999. International trends in rates of hypospadias and cryptorchidism. Environ. Hlth. Perspect. **107:**297–302.

Paulsen CA, Berman NG, Wang C. 1996. Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. Fertil. Steril. **65:**1015–1020.

Peterson R, Theobald H, Kimmel G. 1993. Developmental and reproductive toxicity of dioxins and related compounds: Cross-species comparisons. Critical Reviews in Toxicology **23:**283–335.

Pines A, Cucos S, Ever-Hadani P, Ron M. 1987. Some organochlorine insecticide and polychlorinated biphenyl blood residues in infertile males in the gener-

al Israli population of the middle 1980s. Arch. Environ. Contam. Toxicol. **16:**587–597.

Potashnik G, Goldsmith J, Insler V. 1984. Dibromochloropropane-induced reduction of the sex ratio in man. Androl. **16:**213–218.

Rier SE, Martin DM, Bowman RE, Dmowski WP, Becker JL. 1993. Endometriosis in rhesus monkeys (macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Fund. Appl. Toxicol. **21:**433–441.

Romkes M, Piskorska-Pliszczynska J, Safe S. 1987. Effects of 2,3,7,8-tetrachlorodibeno-*p*-dioxin on hepatic and uterine estrogen receptor levels in rats. Toxicol. Appl. Pharmacol. **87:**306–314.

Sas M, Szollosi J. 1980. Sex ratio of children of fathers with spermatic disorders following hormone therapy. Orv. Hetil. **121:**2807–2808.

Scialli AR, Swan SH, Amler RW, Baird DD, Eskenazi B, Gist G et al. 1997. Assessment of reproductive disorders and birth defects in communities near hazardous chemical sites. II. Female reproductive disorders. Reprod. Toxicol. **11:**231–242.

Scorer CG. 1964. The descent of the testis. Arch. Dis. Child **39:**605–709.

Scott WJ Holson JF. 1977. Weight differences in rat embryos prior to sexual differentiation. J. Embryol. Exp. Morphol. **79:**259–263.

Stachel B, Dougherty RC, Lahl U, Schlosser M, Zeschmar B. 1989. Toxic environmental chemicals in human semen: analytical method and case studies. Andrologia **21:**282–291.

Sutter TR, Guzman K, Dold KM, Greenlee WF. 1991. Targets for dioxin: genes for plasminogen activator inhibitor-2 and interlukin-1b. Science **254:**415–418.

Swan SH, Elkin EP, Fenster L. 1997. Have sperm densities declined? A reanalysis of global trend data. Environ. Hlth. Perspect. **105:**1228–1232.

Tian Y, Ke S, Thomas T, Meeker RJ, Gallo MA. 1998. Transcriptional suppression of estrogen receptor gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). J. Steroid Biochem. Mol. Biol. **67:**17–24.

Tielmans E, Burdorf A, te Velde ER, Weber RFA, van Kooij RJ, Veulemans H, Heederiik DJJ. 1999. Occupationally related exposures and reduced semen quality: a case-control study. Fertil. Steril. **71:**690–696.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJJ, Jegou P, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, SkakkebFk NE. 1996. Male reproductive health and environmental xenoestrogens. Environ. Hlth. Perspect. **104(Suppl. 4):**741–803.

Tsunoda Y Tokunaga T, Sugie T. 1985. Altered sex ratio of live young after transfer of fast- and slow-developing mouse embryos. Gamete Res. **12:**301–304.

Vandenbergh JG, Huggett CL. 1994. Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. Proc. Ntl. Acad. Sci. USA **91:** 11055–11059.

van der Pal Bruin KM, Verloove-Vanhorick SP, Roeleveld N. 1997. Change in male:female ratio among newborn babies in The Netherlands. Lancet **349:**62.

Vercillini P, Oldani S, De Giorgi O, Crosignani PG. 1995. Endometriosis: an overview of descriptive and analytic epidemiological studies, .p. 221–236. *In* Coutinho EM, Spinol P, de Moura LH (ed.), Progress in the management of endometriosis. The Parthenon Publishing Group, New York.

Vierula M, Niemi M, Keiski A, Saarikoski S, Suominen J. 1996. High and unchanged sperm counts of Finish men. Int. J. Androl. **19:**11–17.

Wittmaack FM, Shapiro SS. 1992. Longitudinal study of semen quality in Wisconsin men over one decade. Wis. Med. J. **91:477**–479.

Xu KP, Yadav BR, King WA, Betteridge KJ. 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vivo. Mol. Hum. Reprod. **31:**249–252.

Yang JZ, Yagminas A, Foster WG. 1993. Stimulating effects of 4-chlorodiphenyl ether on surgically induced endometriosis in the mouse. Reprod. Toxicol. **11:**69–75.

Yang JZ, Foster WG. 1997. Continuous exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin inhibits the growth of surgically induced endometriosis in the ovariectomized mouse treated with high dose estradiol. Toxicol. Ind. Health **13:**15–25.

Yang JZ, Agarwal SK, Foster WG. 2000. Subchronic exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin modulates the pathophysiology of endometriosis in the Cynomolgus monkey. Tox. Sci. **56:**374–381.

YoungLai EV, Collins JA, Foster WG. 1998. Canadian semen quality: an analysis of sperm density among eleven academic fertility centres. Fertil. Steril. **70:**76–80.

Zober A, Hoffmann G, Ott MG, Will W, Germann C, van Ravenzwaay B. 1995. Study of morbidity of personnel with potential exposure to vinclozolin. Occup. Environ. Med. **52:**233–41.