

The impact of endocrine disrupters on the female reproductive system

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Over the last decades, many tonnes of man-made chemicals have been produced and released into the environment. Many of these chemical substances have the ability to modulate the action of hormones and are called endocrine disrupters. Cell receptors that have been pure receptors for thousands of years have (due to industrialization), become susceptible to the action of exogenous chemicals. The balance of the endocrine system is very important in the human body especially in females because the menstrual cycle and fertility are very sensitive to hormone imbalances. This review considers the mode of exposure and action of endocrine disrupters and focuses on their impact on the female reproductive system, including female hormone concentrations, menstrual cycle, fertility, spontaneous abortion and the development of endometriosis. An attempt is made to elucidate the impact of endocrine disrupters on the female reproductive system, while admitting that most scientific data come from experimental animals and the conclusions cannot be applied to humans easily. The aim is to present available information, highlighting the impact of endocrine disrupters on the female reproductive system, in order to stimulate re-evaluation in identifying hormone disorders.

Key words: endocrine disrupters/endometriosis/female hormones/pesticides/reproduction

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Introduction

Over the last 50–60 years, many types of man-made chemicals have been manufactured and many of them have become widespread environmental contaminants (Simonich and Hites, 1995; for reviews, see Loganathan and Kannan, 1994; Bjerregaard, 1995). There is now growing concern that some of these man-made chemicals (including pesticides, industrial chemicals, plastics, detergents, paints and cosmetics) are affecting

the health of human and wildlife populations (for reviews, see Loganathan and Kannan, 1994; Fry, 1995; Dich *et al.*, 1997; Longnecker *et al.*, 1997). These substances can affect human health, by upsetting the balance of the endocrine system, and they are known as hormone-disrupting chemicals or endocrine disrupters. As a result of mankind's use of vast quantities of such chemicals, humans and wildlife are continually exposed to endocrine disrupters (O'Shea *et al.*, 1980).

Many of the endocrine disrupting chemicals are organochlorine substances, which means that they contain chemically combined carbon and chlorine. This binding is very strong and resists degradation by normal biochemical and physical processes. Hence, the organochlorines have a long half-life and they accumulate in the environment as persistent organic pollutants (for reviews, see Hendriks *et al.*, 1995; Simonich and Hites, 1995; Tanabe *et al.*, 1998; Fisher, 1999; Muir *et al.*, 1999).

Organochlorines occur naturally in the environment, but only at very low concentrations. About 2000 compounds are known to be produced by living organisms, which contain chlorine or other halogens (bromine, iodine, or fluorine) (Gribble, 1994). Humans and wildlife have not evolved mechanisms or biochemical pathways to detoxify and excrete these chemical substances, so the organochlorines are stored and accumulated in the lipids and fatty tissue (Hall, 1992; Fisher, 1999).

Chronic exposure to endocrine disrupters results in growing concentrations becoming bioaccumulated. The effects observed in wildlife and humans include decreased hatching in fish, birds and turtles; reproductive problems and decreased fertility in mammals (Gilbertson *et al.*, 1991; Fry, 1995; Muir *et al.*, 1999); decreased sperm quality in humans (Carlsen *et al.*, 1992; Bromich *et al.*, 1994; Auger *et al.*, 1995); behavioural abnormalities in birds and mammals, compromised immune system in mammals; and an increase in the incidence of malformations and cancers of male genital tract (for review, see Dich *et al.*, 1997; Longnecker *et al.*, 1997; Palanza *et al.*, 1999). As these substances disrupt the hormonal balance of the body and many of them disrupt the action of oestrogen, their action in the female reproductive system and female fertility is of great interest.

Exposure

Chemicals including many pesticides, polychlorinated biphenyls (PCBs), dioxins, phthalates, lead, mercury and cadmium do not necessarily remain where they are released into the environment but may be transported in water or on air currents throughout the globe (Loganathan and Kannan, 1994). These substances are bioaccumulated and biomagnified, which means that their concentration increases from one trophic level to the next, within the food chain. Humans, some animals and sea mammals, which are on the highest trophic levels, have the highest concentration of endocrine disrupters (Mossner and Ballschmiter, 1997; Fisher, 1999).

Human exposure to endocrine disrupters may occur in a variety of ways, including ingestion of food and water, inhalation of air and skin absorption. For the majority of these chemicals, the major source of exposure is via food (Hall, 1992). For example, >90% of daily intake of PCBs is through food (Theelen *et al.*, 1993). What is remarkable is that the placenta cannot prevent the organochlorine substances from entering the embryonic circulation (Ando *et al.*, 1986; Kanja *et al.*, 1992). The fetus is exposed to endocrine disrupting chemicals (i.e. exogenous hormones) during the period of organogenesis, which depends on hormone balance. After birth, exposure continues via lactation. As organochlorines are lipophilic substances, they are excreted in the breast milk and ingested by the neonate. These substances are detected in breast milk in significant quantities worldwide (Koopman-Esseboom *et al.*, 1994; van Birgelen, 1998), so that the infant has already a burden of endocrine disrupting chemicals within the first months of its life (Patandin *et al.*, 1999).

Mechanisms of action

The endocrine disrupters modulate the hormonal function in the body and, in particular, affect the steroid hormones. Changes in the effective concentrations of hormones can occur if an endocrine disrupter binds to a specific hormone receptor. This chemical substance may then either mimic the hormone or block the normal biological response by occupying the receptor site. Alternatively, endocrine disrupters may be able to react directly or indirectly with the hormone structure to alter its function, change the pattern of hormone synthesis, or modulate the number of hormone receptors and their affinities for specific molecules (for reviews, see Safe *et al.*, 1991; DeRosa *et al.*, 1998;

Sonnenschein and Soto, 1998). Endocrine disrupters have been also shown to modulate the action of thyroid hormones in the body (Cheek *et al.*, 1999; Osius *et al.*, 1999), while there is evidence that some endocrine disrupters may interact with glucocorticoid receptors (Johansson *et al.*, 1998).

A great deal of work has been carried out on the toxicity of dioxins, especially the most potent congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In-vitro and in-vivo studies have revealed that TCDD has anti-oestrogenic properties (Safe *et al.*, 1991; Zacharewski *et al.*, 1994). It exerts its action through binding to a receptor called the aromatic (aryl) hydrocarbon receptor (Ah receptor), which is an intracellular protein. The binding of dioxin or other endocrine disrupters to the Ah receptor causes induction of cytochrome P450 through the transcription of *CYP1A1* gene and other genes that influence basic cellular processes, e.g. growth, differentiation and programmed cell death (Wu and Whitlock, 1992; Schrenk, 1998; Whitlock, 1999).

Endocrine disrupters and disorders in the female reproductive system

The development and the function of the female reproductive tract depends upon hormone concentrations and balance. Endocrine dysfunction may result in many abnormalities, e.g. menstrual cycle irregularities, impaired fertility, endometriosis, and polycystic ovarian syndrome (PCOS). These abnormalities may result from modulation of the concentrations of oestrogens, thecal androgens and thyroid hormones. As endocrine disrupters have the ability to modulate these hormones, it is vital to establish whether they can affect female genital function.

Evidence for the potential effects of endocrine disrupters comes from diethylstilboestrol (DES). The results of the prescription of DES are well known. Women who were exposed *in utero* to DES are at a high risk of developing clear cell adenocarcinoma of the vagina (Herbst and Scully, 1970; Giusti *et al.*, 1995) and DES is also linked with menstrual irregularities, altered uterine structure, infertility, miscarriage, premature births and breast cancer (Giusti *et al.*, 1995). DES is a xeno-oestrogen and many of the endocrine disrupters act in a similar manner. However, conclusions drawn from studies on DES cannot be generalized because DES acted for a limited period of time, while endocrine disrupters act during the whole life, the concentrations of the endocrine disrupters are diverse and they include a variety of substances.

In-vitro studies

Results from in-vitro experiments indicate the effects of endocrine disrupting chemicals on the female reproductive system and suggest a hypothesis for their in-vivo action and their linkage with disease. The expression of Ah receptor in endometrium is necessary for the dioxin and some other endocrine disrupters to exert their action. The *Ah receptor* RNA and protein in endometrium of premenopausal women is expressed in 43% of the endometria studied and have been correlated with the day of the cycle. The maximum expression in endometria is around the time of ovulation and it decreases with increasing age, indicating that women of reproductive age are likely to be more vulnerable (Kuchenhoff *et al.*, 1999). The exposure of human endometrial cells in culture to dioxin increases the induction of expression of

interleukin-1 β and *plasminogen activator inhibitor-2* mRNAs in a dose-dependent manner but, in this study, expression of *CYP1A1* mRNA was not detected and the amount of *Ah receptor* mRNA was decreased dose-dependently (Yang, 1999). This is unlike previous reports, where binding with the Ah receptor usually induces the expression of *CYP1A1* gene (Whitlock, 1999). More pathways of action for dioxin need to be investigated. TCDD suppresses the gene expression of the oestrogen receptor in the ovary, uterus and liver of mice by decreasing its transcription; this response is probably mediated through the Ah receptor (Tian *et al.*, 1998).

Granulosa cells play a significant role during the ovarian cycle and secretion of steroid hormones. The administration of TCDD in culture of granulosa cells significantly decreases oestradiol production. It has been suggested that TCDD might interrupt the endocrine function of human luteinized granulosa cells by blocking the mitotic signal, either directly, or indirectly through the interaction of protein tyrosine kinase/microtubule associated protein 2 (MAP2) kinase and protein kinase signalling (Enan *et al.*, 1996). The effect of the fungicide, methyl-2-benzimidazole-carbamate, on the primary cultures of human ovarian granulosa cells is similar. It has been reported that methyl-2-benzimidazole-carbamate alters centrosome organization during mitosis in dividing granulosa cells. One possible mechanism of action of this agent is the impairment of spindle microtubule dynamics at the centrosome, which results in metaphase arrest and abnormal chromosome organization (Can and Albertini, 1997).

Hexachlorobenzene is another pesticide which is used worldwide. It alters the cell shape of the ovary surface epithelium of cynomolgus monkeys. Many cells become tall, columnar, highly irregular in outline, and show signs of degeneration instead of being squamous to cuboidal, and lying in a single layer. The nuclei migrate toward the apical surface instead of being in the middle. Cytoplasm contains a large number of lysosomes, and numerous vesicles, which may be swollen endoplasmic reticulum. These effects are dose-related (Babineau *et al.*, 1991; Sims *et al.*, 1991).

The function of placenta may also be modulated by endocrine disrupters. The CYP1A1 enzyme is induced and polycyclic aromatic hydrocarbon-related DNA adducts in placental tissue are found in the placenta of pregnant women exposed to organochlorine chemicals (Laguex *et al.*, 1999).

Effects on female hormone production

The organochlorine pesticide hexachlorobenzene (HCB) is a worldwide persistent organic pollutant and has been detected in various tissues and human fluids including serum and ovarian follicular fluid (van der Ven *et al.*, 1992). The exposure of cynomolgus monkeys to HCB (10.0 mg/kg body weight/day) for approximately three menstrual cycles significantly reduces the concentration of oestradiol at ovulation (Foster *et al.*, 1995). Findings on rats indicate that the effect of HCB on steroidogenesis is indirect (Foster *et al.*, 1992).

The effect of TCDD on the ovary of rats appears to be similar. Simultaneous in-vivo experiments in hypophysectomized rats and in-vitro experiments in granulosa cells, theca-interstitial cells and whole ovarian dispersates indicate that TCDD does not alter ovarian steroidogenesis directly (Son *et al.*, 1999). The results are

similar when the effect of TCDD on human luteinized granulosa cells in culture are examined (Heimler *et al.*, 1998). Evaluation of the accumulation of oestradiol in the culture medium after the addition of different quantities of dioxin and androstenedione or pregnenolone indicates that dioxin alters the oestradiol secretion from human luteinized granulosa cells by depletion of androstenedione. The administration of both chorionic gonadotrophin and TCDD in immature female rats alters the concentrations of oestradiol, FSH and LH, while the expected decrease in serum oestradiol concentrations at 60–72 h after chorionic gonadotrophin treatment is not observed (Li *et al.*, 1995a).

Heptachlor is another organochlorine pesticide with endocrine disrupting action. The treatment of rats with heptachlor suppresses progesterone and oestradiol concentrations in blood and reduces the production of oestradiol by the ovarian cells of treated rats, while production of progesterone depends on the dose of heptachlor (Oduma *et al.*, 1995a).

Progesterone concentrations also decrease during early pregnancy in the rabbit after treatment with the pesticide dichlorodiphenyl-trichloroethane (DDT) (Lindenau *et al.*, 1994), while treatment of pregnant cynomolgus monkeys with TCDD results in decreased concentrations of oestradiol, progesterone and chorionic gonadotrophin (Guo *et al.*, 1999).

As far as we know, there are no published papers on the direct effect of endocrine disrupters on the concentrations of female hormones in humans. The previous experiments provide strong evidence that these chemicals may impair the balance of the endocrine system in women. However, women with prenatal exposure to DES had no alteration in hormone concentrations except for the concentration of testosterone in the post-ovulatory and perimenstrual phases of the cycle, as well as in women with irregular menses (Wu *et al.*, 1980). On the other hand, it is known that treatment with contraceptive pills (exogenous hormones) impairs FSH and LH secretion and results in anovulation (Stubblefield, 1996). More epidemiological studies and laboratory experiments need to be performed before final conclusions on the effect of endocrine disrupters on the concentration of female hormones in humans can be drawn.

Menstrual cycle

Endocrine disrupting chemicals may affect the function of oestrogen and progesterone and/or the hypothalamic-hypophysial axes and may alter the natural menstrual cycle, ovulation and fertility. Epidemiological studies in humans have investigated the impact of endocrine disrupting chemicals on the menstrual cycle and there is a great deal of experimental data on the effect of these substances on the cycle of monkeys or oestrus in rodents.

One of the areas in the world most contaminated with persistent organic pollutants is the Great Lakes. People who eat fish from these Lakes are exposed to various chemical substances. An epidemiological study of women who consumed fish from Lake Ontario showed a link between fish consumption, PCB exposure, and a reduction in menstrual cycle length, indicating the possible impact of PCBs through food on menstrual cycle (Mendola *et al.*, 1997).

Rats exposed to organochlorine pesticides with oestrogenic properties including atrazine (Eldridge *et al.*, 1994, Wetzel *et al.*, 1994, Cooper *et al.*, 1996), heptachlor (Oduma *et al.*, 1995b), and

methoxychlor (Chapin *et al.*, 1997) have oestrous irregularities and prolonged duration. The exposure of rats to different concentrations of 3,3',4,4'-tetrachloroazoxybenzene also results in increased oestrous cycle length (van Birgelen *et al.*, 1999). Even after a single dose treatment with TCDD, rats show cycle irregularities, e.g. longer duration of di-oestrous (Li *et al.*, 1995b).

Another oestrogenic chemical substance is 4-tert-octylphenol. Either neonatal exposure or exposure during adulthood of rats to this chemical results in persistent oestrus in the majority of exposed animals (Blake and Ashiru, 1997). There is also a correlation between PCB exposure and oestrus in rats (Sager and Girard, 1994) or cycle of monkeys (Arnold *et al.*, 1993).

According to epidemiological and experimental data, there is strong evidence that exposure to endocrine disrupters is associated with menstrual cycle disorders. However, more epidemiological data is needed to confirm this and to indicate the levels of exposure which result in an effect on menstrual cycle.

Ovulation

It is known that contraceptive pills contain hormones, oestrogen and progestins and that they block normal ovulation. In the same way, endocrine disrupters with oestrogenic properties could block ovulation, if they reach a specific concentration. This would be possible after a critical point of bioaccumulation, either early in the life of sensitive females or even later, resulting in reproductive disorders.

TCDD inhibits ovulation in rats with direct impairment of follicular rupture and not because of an alteration in ovarian steroidogenesis (Son *et al.*, 1999). Although the effects of TCDD on ovulation of rats have been confirmed (Li *et al.*, 1995a,b), its action was found to be via a direct effect on the ovary and on the hypothalamic-pituitary axis (Li *et al.*, 1995a).

The exposure of neonate mice to the organochlorine pesticide, methoxychlor, results in a dose-dependent reduction of ovulated eggs in adult life after ovulation stimulation (Eroschenko *et al.*, 1997). Ovulation defects are also observed in prenatal and neonatal exposed mice to methoxychlor (Chapin *et al.*, 1997), and there are experimental data (Foster *et al.*, 1992; Goldman *et al.*, 1993; Cooper *et al.*, 1994) that link defects of ovulation in animals with exposure to environmental pollutants. In the absence of epidemiological studies on women, we can only assume that these substances may impair ovulation in humans.

Fertility

In many infertile couples, no apparent cause of infertility can be found even after thorough examination. It has been suggested that spermatozoa may have molecular or biochemical disorders resulting in an inability for fertilization, although their mobility and morphology is normal (for reviews, see Sindhu and Guraya, 1989; Tulsiani *et al.*, 1998).

Lindane (γ -hexachlorocyclohexane) is a widely distributed organochlorine pesticide. This pesticide intercalates into the sperm membrane and alters the molecular dynamics of the lipid bilayer (Silvestroni *et al.*, 1997). Lindane in doses as high as that found in female genital tract secretions may inhibit sperm responsiveness to progesterone *in vitro*, which induces the

acrosome reaction at the site of fertilization (Silvestroni and Palleschi, 1999). This could be a cause of infertility in women exposed to lindane.

Exposure to pentachlorophenol (contained in wood preservatives) has also been suggested as playing a role in infertility. The effect of pentachlorophenol in women with endocrine dysfunction may be at the hypothalamic or suprathalamic level, causing mild ovarian or adrenal insufficiency (Gerhard *et al.*, 1999).

There are already some examples of subfertility in wildlife caused by endocrine disrupters. Lake Apopka in Florida is contaminated by a previous extensive spill of dicofol and DDT. During the 1980s there was a progressive decline in the alligator population on the lake, which is presently still continuing. The population is now only a tenth of the size recorded in the 1970s. A study on the alligators (Guillete *et al.*, 1994) found evidence of decreased reproductive ability. Oestrogen concentrations in female juveniles were twice that of alligators from an unpolluted lake and the juveniles exhibited abnormal ovarian structure. There were also adverse effects in male alligators. These data strongly suggest that the endocrine disrupter chemicals affect hormone concentrations and reproduction. Fertility problems in numerous other organochlorine-exposed wild animals, e.g. seals (Rejinders and Brasseur, 1992) and birds (Giesy *et al.*, 1994), have also been recorded.

Other studies indicate adverse effects of organochlorines on the fertility of experimental animals; treatment with 3,3',4,4'-tetrachlorobiphenyl (group of PCB) results in impaired fertility. The animals show a dose-dependent reduced fecundity as well as decreased fertilization after *in-vitro* insemination with spermatozoa (Huang *et al.*, 1998). Similar effects on conception rates are found after treatment of rhesus monkeys with another PCB, aroclor 1254 (Arnold *et al.*, 1995). The presence of aroclor 1254 reduces both the IVF rates and embryonic development in mice (Kholkute *et al.*, 1994a,b,c). However, trace amounts of organochlorine compounds (found in follicular fluid of women undergoing IVF), had no effect on fertilization rates and time to cleavage (Jarrell *et al.*, 1993). Nevertheless, this result does not rule out effects of these organochlorines on fertilization *in vivo*.

The pesticide methoxychlor accelerates the embryo transport rate in rats and induces preimplantation embryonic loss, perhaps due to this acceleration (Cummings and Perreault, 1990). The exposure of rabbits to sparse organochlorine compounds (PCB, DDT, γ -hexachlorobenzene) has almost no effect on fertility but organochlorine compounds are detected in uterine secretions and blastocysts. A small increase in blastocyst loss after PCB exposure may be due to its embryo toxicity (Seiler *et al.*, 1994). The results suggest that organochlorine compounds may have adverse effects on fertilization and some cases of unknown infertility may be explained by the action of these chemical substances.

Spontaneous abortion

The organochlorine compounds entering the embryonic circulation through the placenta could affect the pregnancy outcome resulting in many congenital disorders but also in spontaneous abortion. In southeastern Turkey during 1955–1957, women were accidentally exposed to the pesticide hexachlorobenzene after eating contaminated seed grain and, as a result, developed

porphyria cutanea tarda. There was strong correlation between hexachlorobenzene in serum samples and the risk of spontaneous abortion in those women (Jarrell *et al.*, 1998). However, in another epidemiological study in Italy, no correlation was found between the serum concentration of hexachlorobenzene and spontaneous abortion (Leoni *et al.*, 1986). More than 20% of women with repeated miscarriages have higher concentrations of organochlorines than reference populations. Organochlorines may be responsible for miscarriages in a sensitive population (Gerhard *et al.*, 1998). Another population in southern California potentially exposed to pesticides occupationally or environmentally does not appear to have increased risk for spontaneous abortion but, in contrast, has a lower risk (Willis *et al.*, 1993). PCB concentrations are higher in the blood of women undergoing miscarriage than women at full term (Leoni *et al.*, 1989) or in the second trimester of pregnancy (Bercovici *et al.*, 1983).

One attempt to interpret the mechanism through which PCBs induce spontaneous abortion suggests that arachlor 1242 increases the frequency of contractions of uteri via the calcium and arachidonic acid released by phospholipase A₂ (Bae *et al.*, 1999a,b).

The treatment of cynomolgus monkeys with TCDD during pregnancy alters the concentrations of some hormones and increases the risk of early fetal loss. TCDD may result in endocrine imbalance, which leads to placental insufficiency, compromised embryonic circulation and finally early fetal loss (Guo *et al.*, 1999). It seems that exposure to endocrine disrupting chemicals may increase the incidence of spontaneous abortion in sensitive or more exposed populations. A mechanism through which PCBs may increase spontaneous abortion has been proposed (Bae *et al.*, 1999a,b). The action of other organochlorines on the uterus should also be investigated using quantities similar to those found in the human body.

Endometriosis

Endometriosis is the presence of endometrial tissue (glands and stroma) outside the uterus. The disease occurs naturally only in humans and non-human primates. There is strong evidence that endometriosis is a complex trait in which multiple gene loci interact with each other and the environment to produce the expressed phenotype (Kennedy, 1999). It is an oestrogen-related disease and oestrogens are known to stimulate the growth of endometrial lesions. The medical treatment (oral contraceptives, progestins, gestrinone, danazol and gonadotrophin-releasing hormone agonists) is designed to suppress oestrogen synthesis, thereby inducing atrophy of ectopic endometrial implants or interrupting the cycle of stimulation and bleeding (D'Hooghe and Hill, 1996).

As endometriosis is an oestrogen-related disease and some endocrine disrupters in the human body mimic oestrogen, the link between endometriosis and endocrine disrupters should be investigated. In Belgium, a country heavily polluted with dioxin, the incidence of endometriosis is high (Koninckx *et al.*, 1994). Of course, this does not prove that dioxins are the only cause. Endometriosis is a multifactorial disease and the Belgian population may have other characteristics that promote the development of endometriosis. Very strong evidence comes from a long-term study on the health effects of chronic dioxin exposure

on rhesus monkeys (Rier *et al.*, 1993, 1995). For a period of 4 years between 1977 and 1982, one group of monkeys were fed with 25 parts per trillion (ppt) per day dioxin in their diet, a second group were fed with 5 ppt dioxin and a third group acted as control and were not given dioxin. The animals were examined 10 years after the end of dioxin treatment; five out of seven animals (71%) exposed to 25 ppt dioxin and three out of seven animals (43%) exposed to 5 ppt dioxin had moderate to severe endometriosis compared with 33% of animals in the control group. This is similar to an overall prevalence of 30% in 304 rhesus monkeys with no history of dioxin exposure. The differences were statistically significant ($P < 0.05$) in both groups of exposed animals. It is concluded that chronic exposure to dioxin is directly correlated with a significant increase in the incidence of the development of endometriosis.

According to this experiment, there is strong evidence that dioxin exposure leads to development of endometriosis, but there is no epidemiological evidence from large studies on humans. The population in Seveso, Italy, which was exposed to dioxin after an accident, would be ideal for designing an epidemiological study (Bois and Eskenazi, 1994), but as far as we know, nothing has been published yet. Additional information comes from other studies. It is known that endometriosis is a major factor in female infertility, and that 18% of women with endometriosis have measurable concentrations of TCDD in their blood, while only 3% of women with tubal infertility have measurable concentrations of TCDD.

It is notable that the severity of endometriosis has no direct link with the concentration of dioxin (Mayani *et al.*, 1997). Also, there is no association between mean organochlorine plasma concentrations of 14 polychlorinated biphenyl congeners and 11 chlorinated pesticides in women with endometriosis and a control group of women. Exposure to these organochlorines is not a risk factor for developing endometriosis in the general population; however, the concentrations of these organochlorines were only measured during adulthood and the effect of exposure during uterine life, infancy or puberty is unknown. In this study, the concentrations of organochlorines were low and what might happen at higher concentrations could not be predicted (Lebel *et al.*, 1998).

Endometriosis does not occur naturally in mice and rats, but it can be induced surgically, thus permitting the study of the role of factors in the development and promotion of endometriotic lesions. Perinatal and adult exposure to TCDD can increase the size of endometriotic lesions surgically induced in mice but not in rats (Cummings *et al.*, 1999). 2,3,7,8-TCDD (1 and 3 µg/kg body weight but not 10 µg/kg body weight) and 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF) (100 µg/kg body weight but not in smaller doses) significantly enhance the growth of endometriotic lesions after exposure only during the peri-operative period. In contrast, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 3,3',4,4',5-pentachlorobiphenyl (PCB 126) does not produce a statistically significant increase in endometriotic lesions (Johnson *et al.*, 1997).

The continuous exposure to TCDD of ovariectomized mice results in regression of the endometriotic implants if they were previously treated with a high dose of exogenous oestrogen. These results indicate that oestrogen and dioxin may interact to produce endometriosis or to promote endometriotic lesions (Yang

and Foster, 1997). After the surgical induction of endometriosis in rats, treatment with oestrogen promoted the growth of endometriosis, while treatment with progesterone either produced regression or failed to maintain the sites. Methoxychlor supported the development of endometriosis, at a relatively high dose. These results suggest that exposure of women to high doses of methoxychlor may exacerbate the development of endometriosis or contribute to its recurrence (Cummings and Metcalf, 1995).

Definite conclusions about the effect of dioxin or other organochlorines on the endometriosis cannot be reached from the above results. Endometriosis is a multifactorial disease (Kennedy, 1997). Large studies on the effect of endocrine disrupters on the endometriosis have not been reported. Experiments on animals have not reached any final conclusions and an animal model for studying endometriosis is yet to be developed. It was found that mice and rats respond differently to dioxin (Cummings *et al.*, 1996), with regard to the endometrial site diameter, ovarian function and the immune reaction. Experiments on mice and rats may be of questionable value, since the results may not be applicable to humans. The previously described experimental studies provide evidence that some endocrine disrupters may affect the development and promotion of endometriosis and this should be the conclusion until more evidence is available.

Conclusion

Chemical substances acting as endocrine disrupters exert oestrogenic or anti-oestrogenic activity, which interferes with the function of the highly sensitive female reproductive system. There is not sufficient data concerning humans. The limited results from epidemiological or laboratory studies on women and some more extended results from experimental animals support the hypothesis that endocrine disrupters impair female reproduction. These substances may alter both the hormone concentrations and the menstrual cycle of women as well as their fertility. There is also evidence that endocrine disrupters may enhance the development and promotion of endometriosis, while it would be interesting to elucidate the correlation between PCOS and the endocrine disrupters. Although PCOS is related to hormone irregularities, there is no published study investigating this correlation.

In most studies documented, animals were exposed to a few chemical substances for a certain period of time. On the other hand, humans are exposed concomitantly to a great number of chemical substances over their lifetime. These various chemical substances may enhance or have an antagonistic effect on each other. The accumulated concentrations of the chemicals in animals are also different from that in women. In order to increase the available data and information on the impact of endocrine disrupting chemicals on human health, it has been proposed that a marker of exposure should be developed (Soto *et al.*, 1997). This marker, if developed in a screening test, would estimate the whole exposure, as exposure to individual substances may not be reliable because of the cumulative, synergistic or antagonistic effects among different substances.

It therefore appears urgent that research strategies should focus on the potential effects of endocrine disrupters. Current research investigates infertility problems with no regard for the potential

effects of these relatively new chemical substances. The identification of the exact effects and role of these endocrine disrupters may help explain some cases of unknown infertility.

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References

- Ando, M., Saito, H. and Wakisaka, I. (1986) Gas chromatographic and mass spectrometric analysis of polychlorinated biphenyls in human placenta and cord blood. *Env. Res.*, **41**, 14–22.
- Arnold, D.L., Bryce, F., Stapley, R. *et al.* (1993) Toxicological consequences of Aroclor 1254 ingestion by female rhesus (*Macaca mulatta*) monkeys. Part 1A. Prebreeding phase: clinical health findings. *Food Chem. Toxicol.*, **31**, 799–810.
- Arnold DL, Bryce, F., McGuire, P.F. *et al.* (1995) Toxicological consequences of aroclor 1254 ingestion by female rhesus (*Macaca mulatta*) monkeys. Part 2. Reproduction and infant findings. *Food Chem. Toxicol.*, **33**, 457–474.
- Auger, J., Kuntzmann, J.M., Czyglik, F. *et al.* (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *N. Engl. J. Med.*, **332**, 281–285.
- Babineau, K.A., Singh, A., Jarrell, J.F. *et al.* (1991) Surface epithelium of the ovary following oral administration of hexachlorobenzene to the monkey. *J. Submicrosc. Cytol. Pathol.*, **23**, 457–464.
- Bae, J., Peters-Golden, M. and Loch-Carusio, R. (1999a) Stimulation of pregnant rat uterine contraction by the polychlorinated biphenyl (PCB) mixture aroclor 1242 may be mediated by arachidonic acid release through activation of phospholipase A₂ enzymes. *J. Pharmacol. Exp. Ther.*, **289**, 1112–1120.
- Bae, J., Stuenkel, E.L. and Loch-Carusio, R. (1999b) Stimulation of oscillatory uterine contraction by the PCB mixture Aroclor 1242 may involve increased [Ca²⁺]_i through voltage-operated calcium channels. *Toxicol. Appl. Pharmacol.*, **155**, 261–272.
- Bercovici, B., Wassermann, M., Cucos, S. *et al.* (1983) Serum levels of polychlorinated biphenyls and some organochlorine insecticides in women with recent and former missed abortions. *Environ. Res.*, **30**, 169–174.
- Bjerregaard, P. (1995) Health and environment in Greenland and other circumpolar areas. *Sci. Total. Environ.*, **160/161**, 521–527.
- Blake, C.A. and Ashiru, O.A. (1997) Disruption of rat estrous cyclicity by the environmental estrogen 4-tert-octylphenol. *Proc. Soc. Exp. Biol. Med.*, **216**, 446–451.
- Bois, F.Y. and Eskenazi, B. (1994) Possible risk of endometriosis for Seveso, Italy, residents: an assessment of exposure to dioxin. *Environ. Health Perspect.*, **102**, 476–477.
- Bromich, P., Cohen, J., Steward, I. *et al.* (1994) Decline in sperm counts: an artefact of changed reference range to normal? *Br. Med. J.*, **309**, 19–22.
- Can, A. and Albertini, D.F. (1997) M-phase specific centrosome-microtubule alterations induced by the fungicide MBC in human granulosa cells. *Mutat. Res.*, **373**, 139–151.
- Carlsen, E., Giwercman, A., Keiding, N. *et al.* (1992) Evidence for decreasing quality of semen during the past 50 years. *Br. Med. J.*, **305**, 609–613.
- Chapin, R.E., Harris, M.W., Davis, B.J. *et al.* (1997) The effects of perinatal/ juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundam. Appl. Toxicol.*, **40**, 138–157.
- Cheek, A.O., Kow, K., Chen, J. *et al.* (1999) Potential mechanisms of thyroid disruption in humans interaction of organochlorine compounds with thyroid receptor, transcription, and thyroid binding globulin. *Environ. Health Perspect.*, **107**, 273–278.
- Cooper, R.L., Barrett, M.A., Goldman J.M. *et al.* (1994) Pregnancy alterations following xenobiotic-induced delays in ovulation in the female rat. *Fundam. Appl. Toxicol.*, **22**, 474–480.

- Cooper, R.L., Stoker, T.E., Goldman, J.M. *et al.* (1996) Effect of atrazine on ovarian function in the rat. *Reprod. Toxicol.*, **10**, 257–264.
- Cummings, A.M. and Perreault, S.D. (1990) Methoxychlor accelerates embryo transport through the rat reproductive tract. *Toxicol. Appl. Pharmacol.*, **102**, 110–116.
- Cummings, A.M. and Metcalf, J.L. (1995) Effects of estrogen, progesterone, and methoxychlor on surgically induced endometriosis in rats. *Fundam. Appl. Toxicol.*, **27**, 287–290.
- Cummings, A.M., Metcalf, J.L. and Birnbaum, 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.
- Cummings, A.M., Hedge, J.M. and Birnbaum, L.S. (1999). Effect of prenatal exposure to TCDD on the promotion of endometriotic lesion growth by TCDD in adult female rats and mice. *Toxicol. Sci.*, **52**, 45–49.
- DeRosa, C., Richter, P., Pohl, H. *et al.* (1998) Environmental exposures that affect the endocrine system: public health implications. *J. Toxicol. Environ. Health B. Crit. Rev.*, **1**, 3–26.
- D'Hooghe, T.M. and Hill, J.A. (1996) Endometriosis. In Berek, J.S., Adashi, E.Y. and Hillard, P.A. (eds), *Novak's Gynecology*. 12th edn. Williams & Wilkins, pp. 887–907.
- Dich, J., Zahm, S.H., Hanberg, A. *et al.* (1997) Pesticides and cancer. *Cancer Causes Control*, **8**, 420–443.
- Eldridge, J.C., Fleenor-Heyser, D.G., Extrom, P.C. *et al.* (1994) Short-term effects of chlorotriazines on estrus in female Sprague–Dawley and Fischer 344 rats. *J. Toxicol. Environ. Health.*, **43**, 155–167.
- Enan, E., Moran, F., VandeVoort, C.A. *et al.* (1996) Mechanism of toxic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in cultured human luteinized granulosa cells. *Reprod. Toxicol.*, **10**, 497–508.
- Eroschenko, V.P., Swartz, W.J. and Ford, L.C. (1997) Decreased superovulation in adult mice following neonatal exposures to technical methoxychlor. *Reprod. Toxicol.*, **11**, 807–814.
- Fisher, B.E. (1999) Most unwanted persistent organic pollutants. *Envir. Health Perspect.*, **107**, A18–A23.
- Foster, W.G., Pentick, J.A., McMahon, A. *et al.* (1992) Ovarian toxicity of hexachlorobenzene (HCB) in the superovulated female rat. *J. Biochem. Toxicol.*, **7**, 1–4.
- Foster, W.G., McMahon, A., Younglai, E.V. *et al.* (1995) Alterations in circulating ovarian steroids in hexachlorobenzene-exposed monkeys. *Reprod. Toxicol.*, **9**, 541–548.
- Fry, D.M. (1995) Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ. Health Perspect.*, **103** (Suppl. 7), 165–171.
- Gerhard, I., Daniel, V., Link, S. *et al.* (1998) Chlorinated hydrocarbons in women with repeated miscarriages. *Environ. Health Perspect.*, **106**, 675–681.
- Gerhard, I., Frick, A., Monga, B. *et al.* (1999) Pentachlorophenol exposure in women with gynecological and endocrine dysfunction. *Environ. Res.*, **80**, 383–388.
- Giesy, J.P., Ludwig, J. and Tillit, D.E. (1994) Deformities in birds of the Great Lakes region. *Environ. Sci. Technol.*, **28**, 128–135.
- Gilbertson, M., Kubiak, T., Ludwig, J. *et al.* (1991) Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. *J. Toxicol. Environ. Health*, **33**, 455–520.
- Giusti, R.M., Iwamoto, K. and Hatch, E.E. (1995) Diethylstilbestrol revisited: a review of the long-term health effects. *Ann. Intern. Med.*, **122**, 778–788.
- Goldman, J.M., Stoker, T.E., Perreault, S.D. *et al.* (1993) Influence of the formamidine pesticide chlordimeform on ovulation in the female hamster: dissociable shifts in the luteinizing hormone surge and oocyte release. *Toxicol. Appl. Pharmacol.*, **121**, 279–290.
- Gribble, G.W. (1994) The natural production of chlorinated compounds. *Environ. Sci. Technol.*, **28**, A310–A318.
- Guillete, L.J., Gross, T.S., Masson, G.R. *et al.* (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ. Health Persp.*, **102**, 608–613.
- Guo, Y., Hendrickx, A.G. and Overstreet, J.W. (1999) Endocrine biomarkers of early fetal loss in cynomolgus macaques (*Macaca fascicularis*) following exposure to dioxin. *Biol. Reprod.*, **60**, 707–713.
- Hall, R.H. (1992) A new threat to public health: organochlorines and food. *Nutr. Health*, **8**, 33–43.
- Heimler, I., Rawlins, R.G., Owen, H. *et al.* (1998) Dioxin perturbs, in a dose- and time-dependent fashion, steroid secretion, and induces apoptosis of human luteinized granulosa cells. *Endocrinology*, **139**, 4373–4379.
- Hendriks, A.J., Ma, W.C., Brouns, J.J. *et al.* (1995) Modelling and monitoring organochlorine and heavy metal accumulation in soils, earthworms, and shrews in Rhine-delta floodplains. *Arch. Environ. Contam. Toxicol.*, **29**, 115–127.
- Herbst, A.L. and Scully, R.E. (1970) Adenocarcinoma of the vagina in adolescence: a report of 7 cases including 6 clear-cell adenocarcinoma (so called mesonephromas). *Cancer*, **25**, 745–747.
- Huang, A., Lin, S., Inglis, R. *et al.* (1998) Pre- and postnatal exposure to 3,3',4,4'-tetrachlorobiphenyl: II. Effects on the reproductive capacity and fertilizing ability of eggs in female mice. *Arch. Environ. Contam. Toxicol.*, **34**, 209–214.
- Jarrell, J.F., Villeneuve, D., Franklin, C. *et al.* (1993) Contamination of human ovarian follicular fluid and serum by chlorinated organic compounds in three Canadian cities. *Canad. Med. Assoc. J.*, **148**, 1321–1327.
- Jarrell, J., Gocmen, A., Foster, W. *et al.* (1998) Evaluation of reproductive outcomes in women inadvertently exposed to hexachlorobenzene in southeastern Turkey in the 1950s. *Reprod. Toxicol.*, **12**, 469–476.
- Johansson, M., Nilsson, S. and Lund, B.O. (1998) Interactions between methylsulfonyl PCBs and the glucocorticoid receptor. *Environ. Health Perspect.*, **106**, 769–772.
- Johnson, K.L., Cummings, A.M. and Birnbaum, L.S. (1997) Promotion of endometriosis in mice by polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. *Environ. Health Perspect.*, **105**, 750–755.
- Kanja, L.W., Skaare, J.U., Ojwang, S.B.O. *et al.* (1992) A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs. *Arch. Environ. Contam. Toxicol.*, **22**, 21–24.
- Kennedy, S. (1997) Is there a genetic basis to endometriosis? *Semin. Reprod. Endocrinol.*, **15**, 309–18.
- Kennedy, S. (1999) The genetics of endometriosis. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **82**, 129–133.
- Kholkute, S.D., Rodriguez, J. and Dukelow, W.R. (1994a) Reproductive toxicity of Aroclor-1254: effects on oocyte, spermatozoa, *in vitro* fertilization, and embryo development in the mouse. *Reprod. Toxicol.*, **8**, 487–493.
- Kholkute, S.D., Rodriguez, J. and Dukelow, W.R. (1994b) The effects of polybrominated biphenyls and perchlorinated terphenyls on *in vitro* fertilization in the mouse. *Arch. Environ. Contam. Toxicol.*, **26**, 208–211.
- Kholkute, S.D., Rodriguez, J. and Dukelow, W.R. (1994c) Effects of polychlorinated biphenyls (PCBs) on *in vitro* fertilization in the mouse. *Reprod. Toxicol.*, **8**, 69–73.
- Koninckx, P.R., Braet, P., Kennedy, S.H. *et al.* (1994) Dioxin pollution and endometriosis in Belgium. *Hum. Reprod.*, **9**, 1001–1002.
- Koopman-Elseboom, C., Huisman, M., Weisglas-Kuperus, N. *et al.* (1994) PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. *Chemosphere*, **28**, 1721–1732.
- Kuchenhoff, A., Seliger, G., Klonisch, T. *et al.* (1999) Arylhydrocarbon receptor expression in the human endometrium. *Fertil. Steril.*, **71**, 354–360.
- Lagueux, J., Pereg, D., Ayotte, P. *et al.* (1999) Cytochrome P450 CYP1A1 enzyme activity and DNA adducts in placenta of women environmentally exposed to organochlorines. *Environ. Res.*, **80**, 369–382.
- Lebel, G., Dodin, S., Ayotte, P. *et al.* (1998) Organochlorine exposure and the risk of endometriosis. *Fertil. Steril.*, **69**, 221–227.
- Leoni, V., Fabiani, L., Marinelli, G. *et al.* (1986) Spontaneous abortion in relation to the presence of hexachlorobenzene in the Italian environment. *I.A.R.C. Sci. Publ.*, **77**, 143–146.
- Leoni, V., Fabiani, L., Marinelli, G. *et al.* (1989) PCB and other organochlorine compounds in blood of women with or without miscarriage: a hypothesis of correlation. *J. Occup. Med.*, **35**, 943–949.
- Li, X., Johnson, D.C. and Rozman, K.K. (1995a) Reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in female rats: ovulation, hormonal regulation, and possible mechanism(s). *Toxicol. Appl. Pharmacol.*, **133**, 321–327.
- Li, X., Johnson, D.C. and Rozman, K.K. (1995b) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on estrous cyclicity and ovulation in female Sprague–Dawley rats. *Toxicol. Lett.*, **78**, 219–222.
- Lindenau, A., Fischer, B., Seiler, P. *et al.* (1994) Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. *Hum. Reprod.*, **9**, 772–780.
- Longanathan, B. and Kannan, K. (1994) Global organochlorine contamination trends: an overview. *AMBIO*, **23**, 187–189.
- Longnecker, M.P., Rogan, W.J. and Lucier, G. (1997) The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBs

- (polychlorinated biphenyls) and an overview of organochlorines in public health. *Ann. Rev. Public Health*, **18**, 211–44.
- Mayani, A., Barel, S., Soback, S. *et al.* (1997) Dioxin concentrations in women with endometriosis. *Hum. Reprod.*, **12**, 373–375.
- Mendola, P., Buck, G.M., Sever, L.E. *et al.* (1997) Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. *Am. J. Epidemiol.*, **146**, 955–960.
- Mossner, S. and Ballschmiter, K. (1997) Marine mammals as global pollution indicators for organochlorines. *Chemosphere*, **34**, 1285–1296.
- Muir, D., Braune, B., DeMarch, B. *et al.* (1999) Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Sci. Total Environ.*, **230**, 83–144.
- Oduma, J.A., Wango, E.O., Oduor-Okelo, D. *et al.* (1995a) *In vivo* and *in vitro* effects of graded doses of the pesticide heptachlor on female sex steroid hormone production in rats. *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.*, **111**, 191–196.
- Oduma, J.A., Wango, E.O., Makawiti, D.W. *et al.* (1995b) Effects of graded doses of the pesticide heptachlor on body weight, mating success, oestrous cycle, gestation length and litter size in laboratory rats. *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.*, **110**, 221–227.
- O'Shea, T.J., Brownell, R.L.Jr., Clark, D.R.Jr. *et al.* (1980) Organochlorine pollutants in small cetaceans from the Pacific and south Atlantic Oceans, November, 1968–June, 1976. *Pestic. Monit. J.*, **14**, 35–46.
- Osius, N., Karmaus, W., Kruse, H. *et al.* (1999) Exposure to polychlorinated biphenyls and levels of thyroid hormones in children. *Environ. Health Perspect.*, **107**, 843–849.
- Palanza, P., Morellini, F., Parmigiani, S. *et al.* (1999) Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development. *Neurosci. Biobehav. Rev.*, **23**, 1011–1027.
- Patandin, S., Dagnelie, P.C., Mulder, P.G.H. *et al.* (1999) Dietary exposure to polychlorinated biphenyls and dioxins from infancy until adulthood: a comparison between breast feeding, toddler, and long-term exposure. *Environ. Health Perspect.*, **107**, 45–51.
- Reijnders, P.J.H. and Brasseur, S.M.J.M. (1992) Xenobiotic induced hormonal and associated development disorders in marine organisms and related effects in human; an overview. In Colborn, T. and Clement, C. (eds), *Chemically Induced Alterations In Sexual And Functional Development: The Wildlife/Human Connection*. Princeton Scientific Publishing Co Inc, Princeton, NJ, USA, pp. 159–174.
- Rier, S.E., Martin, D.C., Bowman, R.E. *et al.* (1993) Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam. Appl. Toxicol.*, **21**, 433–441.
- Rier, S.E., Martin, D.C. and Bowman, R.E. (1995) Immunoresponsiveness in endometriosis: implications of estrogenic toxicants. *Environ. Health Perspect.*, **103** (Suppl. 7), 151–156.
- Safe, S., Astroff, B., Harris, M. *et al.* (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds as antioestrogens: characterization and mechanism of action. *Pharmacol. Toxicol.*, **69**, 400–409.
- Sager, D.B. and Girard, D.M. (1994) Long-term effects on reproductive parameters in female rats after translactational exposure to PCBs. *Environ. Res.*, **66**, 52–76.
- Schrenk, D. (1998) Impact of dioxin type induction of drug metabolizing enzymes on the metabolism of endo-xenobiotics. *Biochem. Pharmacol.*, **55**, 1155–1162.
- Seiler, P., Fischer, B., Lindenau, A. *et al.* (1994) Effects of persistent chlorinated hydrocarbons on fertility and embryonic development in the rabbit. *Hum. Reprod.*, **9**, 1920–1926.
- Silvestroni, L., Fiorini, R. and Pallechi, S. (1997) Partition of the organochlorine insecticide lindane into the human sperm surface induces membrane depolarization and Ca²⁺ influx. *Biochem. J.*, **321** (Pt 3), 691–698.
- Silvestroni, L. and Pallechi, S. (1999) Effects of organochlorine xenobiotics on human spermatozoa. *Chemosphere*, **39**, 1249–1252.
- Simonich, S.L. and Hites, R.A. (1995) Global distribution of persistent organochlorine compounds. *Science*, **269**, 1851–1854.
- Sims, D.E., Singh, A., Donald, A. *et al.* (1991) Alteration of primate ovary surface epithelium by exposure to hexachlorobenzene: a quantitative study. *Histol. Histopathol.*, **6**, 525–529.
- Sindhu, K.S. and Guraya, S.S. (1989) Cellular and molecular biology of capacitation and acrosome reaction in mammalian spermatozoa. *Int. Rev. Cytol.*, **118**, 231–281.
- Son, D.S., Ushinohama, K., Gao, X. *et al.* (1999) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) blocks ovulation by a direct action on the ovary without alteration of ovarian steroidogenesis: lack of a direct effect on ovarian granulosa and thecal-interstitial cell steroidogenesis *in vitro*. *Reprod. Toxicol.*, **13**, 521–530.
- Sonnenschein, C. and Soto, A.M. (1998) An updated review of environmental estrogen and androgen mimics and antagonists. *J. Steroid Biochem. Mol. Biol.*, **65**, 143–150.
- Soto, A.M., Fernandez, M.F., Luizzi, M.F. *et al.* (1997) Developing a marker of exposure to xenoestrogen mixtures in human serum. *Environ. Health Perspect.*, **105** (Suppl. 3), 647–654.
- Stubblefield, P.G. (1996) Family planning. I In Berek, J.S., Adashi, E.Y. and Hillard, P.A. (eds), *Novak's Gynecology*. 12th edn. Williams & Wilkins, pp. 227–278.
- Tanabe, S., Senthilkumar, K., Kannan, K. *et al.* (1998) Accumulation features of polychlorinated biphenyls and organochlorine pesticides in resident and migratory birds from South India. *Arch. Environ. Contam. Toxicol.*, **34**, 387–397.
- Theelen, R.M.C., Liem, A.K.D., Slob, W. *et al.* (1993) Intake of 2,3,7,8, chlorine substituted dioxins, furans, and planar PCBs from food in The Netherlands: median and distribution. *Chemosphere*, **27**, 1625–1635.
- Tian, Y., Ke, S., Thomas, T. *et al.* (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.
- Tulsiani, D.R., Abou-Haila, A., Loeser, C.R. *et al.* (1998) The biological and functional significance of the sperm acrosome and acrosomal enzymes in mammalian fertilization. *Exp. Cell Res.*, **240**, 151–164.
- van Birgelen, A.P.J.M. (1998) Hexachlorobenzene as a possible major contributor to the dioxin activity of human milk. *Environ. Health Perspect.*, **106**, 683–688.
- van Birgelen, A.P., Hebert, C.D., Wenk, M.L. *et al.* (1999) Toxicity of 3,3',4,4'-tetrachloroazoxybenzene in rats and mice. *Toxicol. Appl. Pharmacol.*, **156**, 206–221.
- van der Ven, K., van der Ven, H. and Thibold, A. (1992) Chlorinated hydrocarbon content of fetal and maternal body tissues and in pregnant women: a comparison of Germany versus Tanzania. *Hum. Reprod.*, **7** (Suppl. 1), 95–100.
- Wetzel, L.T., Luempert, L.G. 3rd, Breckenridge, C.B. *et al.* (1994) Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. *J. Toxicol. Environ. Health*, **43**, 169–182.
- Whitlock, J.P. (1999) Induction of cytochrome P450A1. *Ann. Rev. Pharmacol. Toxicol.*, **39**, 103–125.
- Willis, W.O., de Peyster, A., Molgaard, C.A. *et al.* (1993) Pregnancy outcome among women exposed to pesticides through work or residence in an agricultural area. *J. Occup. Med.*, **35**, 943–949.
- Wu, C.H., Mangan, C.E., Burtnett, M.M. *et al.* (1980) Plasma hormones in DES-exposed females. *Obstet. Gynecol.*, **55**, 157–162.
- Wu, L. and Whitlock, J.P. Jr. (1992) Mechanism of dioxin action: Ah receptor-mediated increase in promoter accessibility *in vivo*. *Proc. Natl Acad. Sci. USA*, **89**, 4811–4815.
- Yang, J.Z. and Foster, W.G. (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, J.H. (1999) Expression of dioxin-responsive genes in human endometrial cells in culture. *Biochem. Biophys Res. Commun.*, **257**, 259–263.
- Zacharewski, T.R., Bondy, K.L., McDonnell, P. *et al.* (1994) Antiestrogenic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on 17 beta-estradiol-induced pS2 expression. *Cancer Res.*, **54**, 2707–2713.

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