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Review article

The male reproductive system and its susceptibility to endocrine disrupting chemicals

S. Pflieger-Bruss, H.-C. Schuppe and W.-B. Schill

Center of Dermatology and Andrology, Justus Liebig University, Gießen, Germany

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Summary. In the past years, there has been increased interest in assessing the relationship between impaired male fertility and environmental factors. Human male fertility is a complex process and therefore a great variety of sites may be affected by exogenous noxae. Lifestyle factors as well as various environmental and occupational agents may impair male fertility. Many studies have been published reporting on reproductive dysfunctions in male animals and humans. Especially environmental pollutants with endocrine activity are discussed as a possible cause of this detrimental development. Evidence from animal experiments show that substances with oestrogenic and antiandrogenic properties may cause hypospadia, cryptorchidism, reduction of sperm density and an increase of testicular tumours. Many adverse effects on animal male fertility have been documented for phthalates and some chlorinated hydrocarbons such as polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins. For other chemicals such as bisphenol A and nonylphenols animal data are conflicting. Environmental pollutants may mediate their effects by receptor binding, modulation of hormone-regulated mechanisms or direct toxic effects. Data on environmental chemicals and human male fertility are scarce, and risk assessment is mostly based on the results of animal studies. However, there are indications that exposure to endocrine active chemicals during early development may alter hormone responsiveness in adulthood. Furthermore, some of the chemicals are found in fluids that are associated with human reproduction, such as follicular fluid, seminal fluid and cervical mucus. Recent studies suggest a correlation between pesticide exposure and standard semen parameters as well as *in vitro* fertilization rates.

Correspondence: Dr Sybille Pflieger-Bruss, Center of Dermatology and Andrology, Justus Liebig University, Gaffkystr. 14, 35385 Gießen, Germany. Tel.: +49 641 99 43220; Fax: +49 641 99 43259; E-mail: sybille.pflieger-bruss@derma.med.uni-giessen.de

Introduction

In the past years, there have been many reports about reproductive dysfunction in male humans and animals (Guillette *et al.*, 1994, for review see Jørgensen *et al.*, 2001). An increase of human males suffering from cryptorchidism, hypospadia and testicular tumours (Osterlind, 1986; Jackson, 1988) was documented. Environmental pollutants with endocrine activity, so called 'endocrine disruptors', are especially discussed as a possible cause of this detrimental development (Sharpe, 1995). Endocrine disrupting substances may interfere with the production, secretion, transportation, metabolism, receptor binding, mediation of effects, and excretion of natural hormones which regulate developmental processes and support endocrine homeostasis in the organism (Kavlock *et al.*, 1996). Today we distinguish chemicals with anti-oestrogenic oestrogenic, antigestagenic and antiandrogenic effects (Table 1).

Many animal studies have shown that *in utero* or perinatal exposure to xeno-oestrogens [diethylsilbestrol (DES), bisphenol A, mono-n-butylphthalate] or antiandrogens (flutamide, vinclozolin, p,p'-DDE, DDT) may cause hypospadia, cryptorchidism, reduced sperm counts and testicular tumour in males (Skakkebaek *et al.*, 2001). Such clear effects of endocrine disrupting chemicals on reproductive organs have not yet been shown for humans. However, some studies support the hypothesis that exposure to endocrine disruptors may alter human

Table 1. Some substances with endocrine properties

Anti-oestrogenic	Oestrogenic	Antigestagenic	Antiandrogenic
• Dibenzo-p-dioxin	• Isoflavones	• Carbamate	• Vincolzolin
• Coplanar PCBs	• Phthalates		• p,p'-DDE
• Tributyltin	• Ortho-substituted PCB		• Methoxychlor
	• o,p'-DDT, o,p'-DDE		• Dibenzo-p-dioxin
	• Bisphenol A		• Flutamide
	• Alkylphenols		• Linuron
			• Natural pyrethrin
			• Tris(4-chlorophenyl)-methanol

male fertility (Tielemans *et al.*, 1999; Dallinga *et al.*, 2002).

Infertility is defined as a failure to achieve pregnancy while in a stable relationship and engaging in sexual intercourse without contraception for a period of 1 year or longer. Today 15–20% of young couples in the industrialized countries are involuntarily childless (Beier, 1988). However, the majority of these couples will conceive a child in the long-term because of either spontaneous pregnancies or artificial reproductive techniques. In Europe many couples decide to have children when they are 30 years or older, at a time when their natural fertility starts to decline (Dunson *et al.*, 2004). Possible adverse reproductive effects because of environmental contaminant exposure may result in an additional reduction of fertility. Alterations in the male reproductive tract may be transient or permanent, depending on the developmental stage (e.g. foetal life, puberty or adulthood) and the duration of exposure. In general, many different environmental factors such as occupation, infection, pharmacons, radiation (Rowley *et al.*, 1974), temperature (Jung *et al.*, 2001), stress, environmental chemicals (Pflieger-Bruss *et al.*, 1999a), nicotine (Marshburn *et al.*, 1989; Osser *et al.*, 1992; Zavos *et al.*, 1998) and alcohol (Marshburn *et al.*, 1989) may contribute to impaired male fertility. Only for some agents there is proven evidence of their reproductive toxicity. Toxic effects on reproduction are mainly investigated in animal models. However, it is difficult to transfer these results to the human situation. It is most important to carefully document the medical, social and occupational history of andrological patients to discover possible exposure risks. Many different endpoints such as onset of puberty, libido, sexual performance, spermatogenesis, hormonal status, and sperm function are relevant in male reproductive toxicology and have to be considered. Epidemiological studies on environmental and occupational medicine in both exposed and unexposed men have to be collected according to standardized protocols in order to identify or confirm potential reproductive hazards.

Is there a decline in semen quality?

In earlier times, a value of 60×10^6 spermatozoa ml^{-1} was considered to be normal, later 40×10^6 spermatozoa ml^{-1} (Eliasson *et al.*, 1970), and today 20×10^6 spermatozoa ml^{-1} according to World Health Organization (2000). Carlsen *et al.* (1992) reported on declining sperm concentrations in normal men over the past 50 years. The study was based on a meta-analysis of 61 publications between 1938 and 1990. Sperm concentrations dropped from 113 to 66 million ml^{-1}. However, this result is controversial because of methodological problems with the statistical evaluation, unbalanced geographical distribution of the studies included, unrepresentative populations and missing standardized semen analysis (Lerchl & Nieschlag, 1996; Larsen *et al.*, 1998). In the meantime, many retrospective studies have either supported or refuted the findings of Carlsen *et al.* (1992) (for review see Jørgensen *et al.*, 2001). Recent well-designed studies have shown large regional differences in semen quality and time trends, both within and between countries (for review see Jørgensen *et al.*, 2001). The final answer to this question remains to be found. Humans have not only been exposed to endocrine disruptors, they rather have had a tremendous change in their lifestyle during the past 50 years. Even if there has been a deterioration of overall semen quality it is not necessarily because of endocrine disruptors. In farm animals such as bulls, boars and sheep, no decline of sperm counts was shown between 1932 and 1995 (Setchell, 1997).

Methodological difficulties in male reproductive toxicology

Reproductive toxins may exert their adverse effects on pre-testicular, testicular or post-testicular sites.

The hypthalamus-hypophysis-testis axis may be affected as well as the pulsatile secretion of gonadotrophin-releasing hormone and/or gonadotrophins. Furthermore, toxins may impair spermatogenesis directly or indirectly. Direct impairment may include cytotoxic or genotoxic effects on the different spermatogenic cells. The testicular germinal epithelium has a very high proliferation rate and is considered resistant to many toxic agents. This may be partially because of the blood-testis barrier, which regulates the passage of molecules. However, the blood-testis-barrier does not protect spermatogonia, which are located in the basal compartment of the germinal epithelium. In case of the passage of harmful molecules through the blood-testis barrier or its destruction, toxic agents may have free access to all cells of the spermatogenic cycle. Permanent damage of the germinal epithelium is commonly found in cases of intense or prolonged damaging conditions (e.g. alkylating antineoplastic agents, dibromochloropropane (DBCP).

Alterations in Leydig cells functions, the blood-testis barrier and the testicular vascular system as well as reactions of the local immune system may be considered as indirect effects resulting in a reduced fertility. To date, little is known about substances that interfere with post-testicular parameters such as seminal fluid, epididymis, sperm maturation and sperm transport. It is obvious that reduced fertility is often caused by multiple factors. There is no single marker to measure fertility. Parameters such as 'time to pregnancy' cannot be considered to evaluate male fertility as female factors are included. If standard semen parameters are impaired, it is impossible to draw conclusions on the pathomechanism involved. Under physiological conditions, the number, motility and morphology of human spermatozoa vary interindividually and intraindividually and depend on factors such as sexual abstinence. Different *in vivo* animal models have been established to discover possible reproductive toxicants, e.g. chemicals or drugs. However, the results cannot be simply transferred to humans, as many parameters are specific to one species. To evaluate disruptions in spermatogenesis it is of major importance to consider these differences. For instance, rats produce and ejaculate far more spermatozoa than humans and therefore remain fertile even if sperm counts are reduced by 90% (Mably *et al.*, 1992). Partial functions of male fertility can be investigated with *in vitro* models such as primary cell culture of Sertoli cells or Leydig cells, immortalized cell lines or whole organ culture such as testis perfusion, culture of tubuli seminiferi or sperm function tests with epididymal or testicular spermatozoa.

Environmental pollutants with endocrine activity

Alkylphenols

Alkylphenols (AP) consist of a phenol group and an alkane. The compounds may vary both in the relative position of the alkane group and in the length and branching of the alkane. Commercially available alkylphenols are generally mixtures of alkylphenols with different degrees of branching but with the same number of carbon atoms in the chain.

The 4-nonylphenol (para-nonylphenol, NP) is commercially the most prevalent of the alkylphenol family, representing approximately 85% of the alkylphenol market (Fig. 1).

Alkylphenols are mainly used in the production of alkylphenol ethoxylates (APEs), tris(nonylphenyl)phosphite (TNPP) and alkylphenol-formaldehyde condensation resins. There is a wide distribution of these persistent industrial chemicals. They are used as surface active agents in cleaning/washing agents, paints, cosmetics, and even spermicides (Schäfer *et al.*, 1996). NP has also been found in the preparation of lubricating oil additives, plasticizers, and polyvinyl chloride (PVC) used in the food processing and packaging industries. The yearly worldwide production of APEs in 1995 was over 300 000 tons (Jobling *et al.*, 1995). Especially NP bioaccumulates in aquatic organisms (Ahel *et al.*, 1993). In the absence of measured body burden data, the EU commission suggested in their risk assessment report on 4-nonylphenol and nonylphenol (2002) an overall exposure to nonylphenols from food packaging materials of 140 μg day^{-1} (equivalent to 2 μg kg^{-1} day^{-1}). However, these data were based on American residue data and patterns of consumption. Recently it has been shown that nonylphenols are indeed present in different foodstuffs. Based on these results an oral daily intake of 7.5 μg day^{-1} was suggested for the German population (Guenther *et al.*, 2002). So far, data on reproductive toxicology of NP are only available from animal studies or results from *in vitro* test systems. 4-nonylphenol is a weak oestrogen. In summary, *in vitro* and *in vivo* oestrogenic data from the recombinant yeast assay (Routledge & Sumpter, 1997), the MCF-7 cell culture (Soto *et al.*, 1991),

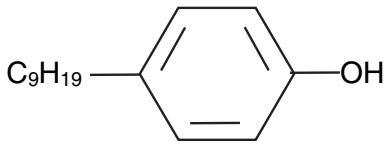

Figure 1. Structure of 4-nonylphenol.

and the uterotrophic response assay (Odum *et al.*, 1997) provides evidence that NP has oestrogenic activity of three to six orders of magnitude less potent than 17β-oestradiol (EU risk assessment report, 2002). The effects of nonylphenol on male fertility were investigated in multigeneration studies and repeated exposure studies. Neonatal rats, exposed to 8 mg kg^{-1} day^{-1} by intraperitoneal injection, showed decreased weights of the reproductive organs and delayed testes descent (Lee, 1998). However, Odum & Ashby (2000) did not confirm these findings in their study. de Jager *et al.* (1999a) exposed adult male rats intragastrically to 100, 250 and 400 mg kg^{-1} day^{-1} for one complete spermatogenetic cycle. Animals exposed to 100 mg kg^{-1} day^{-1} displayed adversely affected seminiferous tubules. The highest dose group showed impaired testicular mass and sperm counts. Other studies also supported the NP toxicity on testis and epididymis in male rats exposed after gestation and early postnatal life (de Jager *et al.*, 1999b, 2001). In a multigeneration study with outbred CB-1 adult mice, exposed to 50 and 500 μg l^{-1} drinking water over 4 weeks (parent generation), and over gestation, lactation, prepubertal, pubertal, and adult life (F1 generation) acrosomal integrity was decreased in both treatment groups of P- and F1-generation (Kyselova *et al.*, 2003). Adeoya-Osiguwa *et al.* (2003) reported on significant stimulation of capacitation and acrosome reaction of mice spermatozoa *in vitro* by 0.001–1 μmol l^{-1} nonylphenol after short-time incubation. It remains to be determined whether the effects seen in these animal models are of any relevance to human male fertility.

Bisphenol A

Bisphenol A is used for many purposes in modern society, e.g. as a chemical building block in the production of polycarbonate plastic and epoxy resins. In Germany the yearly production of this chemical is approximately 210 000 tons. Bisphenol A-based polycarbonate is used as a plastic coating for children's teeth to prevent cavities, as a coating in metal cans to prevent the metal from contact with food contents, as the plastic in food containers, refrigerator shelving, baby bottles, water bottles, returnable containers for juice, milk and water, micro-wave ovenware, artificial teeth, nail polish, compact discs, electric insulators, and as parts of automobiles, certain machines, tools, electrical appliances, and office automation instruments. As the plastic ages, bisphenol A may leach into the environment (Fig. 2).

The weak oestrogenic activity of bisphenol A has been known for a long time, however, the

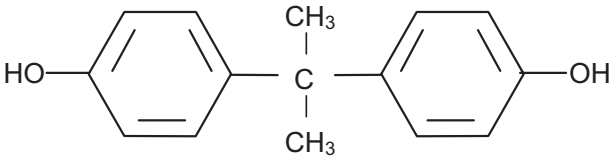

Figure 2. Structure of bisphenol A.

oestrogenic potency of bisphenol A is about 10-fold less than that of 4-nonylphenol (Villalobos *et al.*, 1995). In addition, the oestrogenicity of bisphenol A *in vivo* is dependent on the route of administration. In rats, oral bioavailability of bisphenol A is low and it is mainly transformed to a nonoestrogenic bisphenol A glucuronide in the liver (Pottenger *et al.*, 2000). Nevertheless, excretion in the rat is low because bisphenol A glucuronide is transported into the bile and enters the enterohepatic circulation. In humans, on the other hand, bisphenol A is glucuronided and immediately excreted by the kidneys (Völkel *et al.*, 2002).

Until recently, human exposure data has been missing. Schönfelder *et al.* (2002) measured bisphenol A in the blood of pregnant women, in umbilical blood at birth and in placental tissue. Concentrations ranged from 0.3 to 18.9 ng ml^{-1} in the maternal plasma, 0.2–9.2 ng ml^{-1} in the foetal plasma, and 1.0–104.9 ng g^{-1} in the placental tissue.

Effects of bisphenol A on male reproduction have been controversial. However, there is evidence that bisphenol A binds to the oestrogen receptor (ER) and is able to induce ER-mediated gene expression (Matthews *et al.*, 2001). Disruption and alteration of gene activity during development may alter hormone-responsive tissues in the adult. In rats, bisphenol A exposure of 2.4 μg kg^{-1} day^{-1} from postnatal day 21–35 suppressed serum luteinizing hormone (LH) levels and testosterone levels. Additionally, oestrogen levels were reduced because of the inhibition of aromatase activity in Leydig cells (Akingbemi *et al.*, 2004a). Male rats perinatally exposed to 2.4 μg kg^{-1} bisphenol A had decreased testosterone levels in the testicular interstitial fluid in adulthood (Akingbemi *et al.*, 2004a).

In contrast, postnatal exposure of male rats to 300 μg bisphenol A per kilogram bodyweight had no effect on reproduction (Nagao *et al.*, 1999). Ema *et al.* (2001) conducted a two-generation study to evaluate low-dose effects (0.2, 2, 20, 200 μg kg^{-1} day^{-1}) of bisphenol A in rats. There were no compound-related changes in epididymal sperm counts and sperm motility in F0 and F1 generation. In contrast, 100 ng bisphenol A kg^{-1} daily for 28 days reduced testicular and epididymal sperm counts in mice. Likewise, absolute weights of testes and seminal vesicles were reduced in this treatment group

(Al-Hiyasat *et al.*, 2002). Similar results were reported by Sakaue *et al.* (2001) for adult rats even at very low levels of bisphenol A. Taken together, human risk assessment is very difficult, especially for chronic low dose exposure levels. The doses used in some animal studies are even lower than those found in pregnant women. Therefore, further investigations are urgently needed.

Phthalates

Phthalates [butylbenzylphthalate (BBP), di-n-butylphthalate (DBP), di(2-ethylhexyl)phthalate (DEHP)] are primarily used as plasticizers in PVC products and have attracted much attention because of the fact that especially DEHP is a constituent of infant toys, indoor constructions, food packaging products, and biomedical devices.

The DEHP increases polymer flexibility by attenuating inter-molecular attraction forces. This is accomplished by the embedment of phthalate molecules into the polymer matrix. Accordingly, DEHP is not chemically bound to the polymer and, therefore, is readily released into the environment – despite of its relatively low vapour pressure and low water solubility. Therefore, phthalates are ubiquitous in the environment (Thomas & Thomas, 1984; Fig. 3).

In Western industrialized countries, the yearly production approximates 1–4 million tons. The estimated daily intake of the general population is about 30 μg kg body weight^{-1} (Doull *et al.*, 1999). Consequently, DEHP can be found in animal and human tissues. Latini *et al.* (2003) reported on human *in utero* exposure to DEHP/mono(2-ethylhexyl)phthalate (MEHP) and the duration of pregnancy. There was a significant correlation between MEHP-positive newborns and low gestation age. High concentrations of DEHP have been measured in blood of patients undergoing dialysis, caused by migration of the plasticizer from plastic medical devices (Pollack *et al.*, 1985; Mettang *et al.*, 1996).

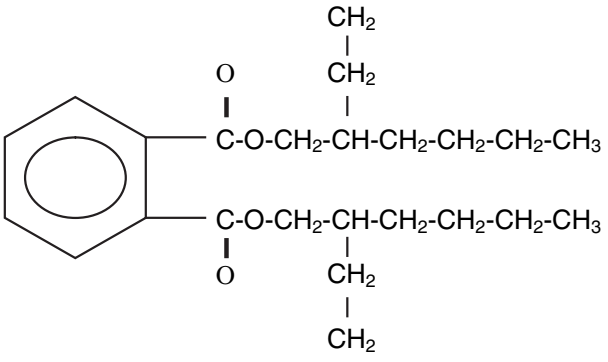

Figure 3. Structure of di(2-ethylhexyl)phthalate.

After oral application DEHP rapidly breaks down to MEHP and 2-ethylhexanol. The breakdown is slower if DEHP enters the blood directly. The major part of DEHP and its metabolites are excreted within 24 h in urine and faeces. Oestrogenic activity for BBP and DBP was shown *in vitro*, but not *in vivo* (Jobling *et al.*, 1995). For DEHP no oestrogenicity has been demonstrated so far. Nevertheless, its reproductive toxicity has been documented in many animal studies. The toxic responses of DEHP *in vivo* seem to be mediated through its first metabolite MEHP. *In utero* exposure to BBP, DBP and DEHP inhibits the differentiation of androgen-sensitive organs. The effects are comparable to the findings seen after exposure to antiandrogens such as vinclozolin and flutamide. DEHP has been shown to alter the expressions of genes that are involved in testis development and steroid hormone synthesis (Wong & Gill, 2002).

Sertoli cells as well as Leydig cells are targets for DEHP and MEHP. Rodent pubs exposed via diet to DEHP *in utero* showed vacuolization of Sertoli cells, and atrophy of the seminiferous tubules (Poon *et al.*, 1997). The most sensitive marker for DEHP toxicity is the immature male reproductive tract. Low-dose chronic DEHP exposure (0, 10, or 100 mg kg^{-1} day^{-1}) during postnatal life up to adulthood induced high serum levels of LH, testosterone and 17β-oestradiol (Akingbemi *et al.*, 2004b). At the same time DEHP elevates Leydig cell proliferative activity and Leydig cell hyperplasia. Persistent elevation of LH and testosterone may increase the risk of precocious puberty and testicular tumours (Laue *et al.*, 1995; Martin *et al.*, 1998).

The MEHP is the metabolite responsible for the effects seen. Li *et al.* (1998) co-cultured neonatal Sertoli cells and gonocytes with micromolar concentrations of MEHP. Normal adhesion of gonocytes to Sertoli cells was disrupted. Additionally, Sertoli cell proliferation was suppressed. In contrast, DEHP caused no such effects under the same *in vitro* conditions (Li *et al.*, 1998). A two-generation study with rats, exposed to 650 mg DBP kg body weight^{-1} day^{-1}, revealed no reduced fertility in the parent generation. However, the F1-generation, which had obtained the same dosage of DBP, showed decreased testis weights, crytorchidism, reduced sperm counts, and malformations of the epididymis and penis (Wine *et al.*, 1997). The exact toxicological mechanism on the male reproductive tract needs to be discovered.

Chlorinated hydrocarbons

Among all environmental pollutants organochlorine compounds are of major interest because of

their long retention time in the organism and the fact that some of these compounds are classified as 'endocrine disruptors'. Organochlorine compounds comprise a large group of substances, e.g. DDT and metabolites, gamma-hexachlorocyclohexane (gamma-HCH), polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs). Human risk assessment is very difficult because these compounds have a variety of toxic effects and humans are usually exposed to a mixture of them. Although the production and application of DDT, PCBs and related compounds has been banned in most industrialized countries, there are still universally found in biological samples. PCB concentrations in biological samples have decreased worldwide in the past 20 years (Kimbrough, 1995). Nevertheless, there is concern about human exposure because PCBs are present in capacitators and transformers that are still operating. Additionally, PCBs are formed *de novo* through incineration (Hagenmaier *et al.*, 1995). Organochlorines preferentially bioaccumulate in the adipose tissue. However, DDT and metabolites, gamma-HCH, PCBs and PCDDs are also found in fluids of the human reproductive tract, such as seminal fluid, cervical mucus, and follicular fluid (Schlebusch *et al.*, 1989; Wagner *et al.*, 1990; Hanf *et al.*, 1995; Dallinga *et al.*, 2002). The main exposure route for DDT, PCBs and related compounds is the consumption of meat, milk and dairy products. For infants breastfeeding is the main exposure route, although levels of PCBs and PCDDs/PCDFs in human milk have decreased to a great extent in the past years. The intake via inhalation and the dermal exposure can be neglected for most individuals.

Dermal exposure to gamma-HCH (lindane) is of some relevance because it is used in the therapy of ectoparasitic diseases. The clinical use of lindane for the treatment of scabies and pediculosis has become somewhat controversial. Lindane is available as a series of preparations in different galenic formulations and in various concentrations. In Europe a 0.3% lindane formulation is used. According to the WHO, the accepted daily intake for lindane is 0.01 mg kg^{-1} day^{-1} (Surber & Rufli, 1995). During the eighties, the lindane concentration in serum of unexposed individuals was approximately 3 ng ml^{-1}. Lindane serum concentrations of 425 ng ml^{-1} were reported after scabies treatment of men with severe skin lesions (Surber & Rufli, 1995). Data on chlorinated hydrocarbons and human male fertility are scarce or controversial. Most of the available data are from animal models.

The effects of PCBs on male reproduction *in vivo* comprise impaired fertility in postnatally exposed rats, reduced matings (rat), decreased concentration of testicular spermatozoa (mouse), lowered weight of the ventral prostate (rat) and seminal vesicles (rat, mouse) (Ahlborg *et al.*, 1992). Single nonortho and mono-ortho PCB as well as 2,3,7,8-TCDD may impair spermatogenesis of rats and marmoset monkey (*Callithrix jacchus*) *in vivo* and *in vitro* (Rune *et al.*, 1991a,b; Pflieger-Bruss *et al.*, 1999b). Especially Sertoli cells and early spermatids show morphological alterations (Rune *et al.*, 1991a; Pflieger-Bruss *et al.*, 1999b). Raychoudhury *et al.* (2000) concluded that the dioxin-like 3,3′,4,4′-teterchlorobiphenyl (PCB 77) directly affects Sertoli cells. No morphological lesions of marmoset monkey and rat Leydig cells were obvious after 2,3,7,8-TCDD exposure. However, histochemically 3β-hydroxysteroid dehydrogenase activity (3β-HSD) was reduced in both species. Male mice exposed to high doses of 2,3,7,8-TCDD for 8 weeks did not show effects on fertility and their offspring revealed no abnormalities (Lamb & Moore, 1981). *In utero* and lactational TCDD exposure reduced sperm production in male rats but did not affect fertility (Mably *et al.*, 1992). This is not contradictory, because rats produce far more spermatozoa than are necessary for their unrestricted fertility.

Male rats exposed to Aroclor 1242 (commercial PCB mixture, Monsanto, St Louis, MO, USA) during lactation showed elevated testis weights, increased daily sperm production, and increased Sertoli cell numbers per testis (Kim, 2001).

Exposure to lindane during lactation reduced testicular weights and the number of spermatozoa and spermatids at adulthood in male rat offspring (Dalsenter *et al.*, 1997).

In humans a correlation between standard semen parameters (motility, morphology, sperm count) and the concentration of PCB and gamma-HCH in the seminal plasma has not been shown (Ensslen *et al.*, 1990). However, Dallinga *et al.* (2002) reported on significantly decreased sperm counts in relation to an elevated PCB metabolite level within a subgroup of men with normal semen quality. Rozati *et al.* (2002) detected PCBs in the seminal fluid of infertile men, but not in the control group of fertile men. Sperm functions are necessary for a normal fertilizing capacity of spermatozoa. Sperm function parameters comprise motility, vitality, acrosome reaction, penetration through the cumulus, binding to the zona pellucida and subsequent fusion with the oocyte membrane. In mammals, including humans, spermatozoa must undergo a number of changes before they gain their fertilizing ability. During the passage of spermatozoa through the female reproductive tract several biochemical changes take place in the plasma membrane, which enable them to undergo the acrosome reaction. Prematurely acrosome-reacted spermatozoa lose their fertilizing

potential (Cherr *et al.*, 1986). Fertilization rates in mouse *in vitro* fertilization (IVF) procedures were decreased when capacitated mouse spermatozoa had been incubated with PCB-exposed oocytes (Kholkute *et al.*, 1994a). In contrast, the fertilizing capacity of PCB-treated spermatozoa was unaffected when they had been incubated with untreated mouse oocytes (Kholkute *et al.*, 1994b). No effect of 3,3′ 4,4′-tetrachlorobiphenyl (PCB 77) on the motility of human spermatozoa *in vitro* was found, although PCB 77 was bioavailable as shown by analytic procedures (Hanf *et al.*, 1995). Earlier Hanf *et al.* (1992) examined the effects of different PCDD congeners on human sperm motility *in vitro*. 2,3,7,8-TCDD as well as the other PCDD congeners had no effect on human sperm motility. The 2,3,7,8-TCDD concentration in the medium was about 10 000-fold above the average level in body fluids. In contrast, Roediger *et al.* (1989) demonstrated that human sperm motility decreased compared with controls after exposure to low concentrations of 2,2′,6,6′-tetrachlorobiphenyl. Additionally, the induction of the acrosome reaction was reported (Roediger *et al.*, 1989). Data from our own laboratory suggest that very high concentrations of PCB 77, 126, 153 and 118 do not affect the motility and vitality of human spermatozoa *in vitro* (Pflieger-Bruss *et al.*, 1999a). After human spermatozoa had been exposed to different single PCB congeners during capacitation, the spontaneous acrosome reaction was determined. No significant differences were found between PCB-exposed spermatozoa and the control group (Pflieger-Bruss *et al.*, 1999a). Nevertheless, negative effects on sperm function cannot be totally excluded as other organohalogen compounds and heavy metals can be identified in the genital tract and their synergistic or additive effects are mostly unknown.

Tielemans *et al.* (1999) suggested that pesticide exposure decreases human sperm fertilizing ability *in vitro*. Fertilization rates in the IVF treatment were significantly decreased for couples with male partners occupationally exposed to pesticides (Tielemans *et al.*, 1999). However, the authors did not draw conclusions as to which chemical may be responsible for that effect. More human data on the effects of environmental factors on human male fertility is urgently needed to facilitate risk assessment.

References

Adeoya-Osiguwa SA, Markoulaki S, Pocock V, Milligan SR, Fraser LR (2003) 17β-estradiol and environmental estrogens significantly affect mammalian sperm function. Hum Reprod 18:100–107.

Ahel M, McEvoy J, Giger W (1993) Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. Environ Pollut 79:243–248.

Ahlborg UG, Hanberg A, Kenne K (1992) Risk Assessment of Polychlorinated Biphenyls. Institute of Environmental Medicine, Karolinska Institute, Stockholm, Schweden.

Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP (2004a) Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitariy luteinizing hormone secretion and decreased steroidogenetic enzyme gene expression in rat Leydig cells. Endocrinology 145:592–603.

Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP (2004b) Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci USA 101:775–780.

Al-Hiyasat AS, Darmani H, Elbetieha AM (2002) Effects of bisphenol A on adult male mouse fertility. Eur J Oral Sci 110:163–167.

Beier HM (1988) Umweltbelastungen als Störungen der Frühgravidität und als Abortursache. Gynäkologe 21:245–248.

Carlsen E, Giwercman A, Keidinger N, Skakkebaek NE (1992) Evidence for decreasing quality of semen during past 50 years. BMJ 305:609–612.

Cherr GN, Lambert H, Meizel S, Katz DF (1986) *In-vitro* studies of the golden hamster sperm acrosome reaction: completion on the zona pellucida and induction by homologous soluble zonae pellucidae. Dev Biol 114:119–131.

Dallinga JW, Moonen EJC, Dumoulin JCM, Evers JLH, Geraedts JPM, Keinjans JCS (2002) Decreased human semen quality and organochlorine compounds in blood. Hum Reprod 17:1973–1979.

Dalsenter PR, Faqi AS, Webb J, Merker HJ, Chahoud I (1997) Reproductive toxicity and toxicokinetics of lindane in the male offspring of rats exposed during lactation. Hum Exp Toxicol 16:146–153.

Doull J, Cattley R, Elcombe C, Lake BG, Swenberg J, Wilkinson C, van Williams G Gemert M (1999) A cancer risk assessment of di(2-ethylhexyl)phthalate: application of the new U.S.EPA risk assessment guidelines. Regul Toxicol Pharmacol 29:327–357.

Dunson DB, Baird DD, Colombo B (2004) Increased infertility with age in men and women. Obstet Gynecol 103:51–56.

Eliasson G, Hellinga F, Lübcke F, Meyhöfer H, Niermann H, Steemp O, Schirren C (1970) Empfehlungen zur Nomenklatur in der Andrologie. Andrologie 2:286.

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A (2001) Rat two-generation reproductive toxicity study of bisphenol A. Reprod Toxicol 15:505–523.

Ensslen SC, Riedel HH, Bluthgen H, Heeschen W (1990) Chlorkohlenwasserstoff im Seminalplasma und menschliche Fertilität. Zbl Gynäkol 112:817–821.

Guenther K, Heinke V, Thiele B, Kleist E, Prast H, Raecker T (2002) Endocrine disrupting nonylphenols are ubiquitous in food. Environ Sci Technol 36:1676–1680.

Guillette LJ, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688.

Hagenmaier H, Krauss P, Lindig C (1995) Herkunft und Verbleib von Dioxinen, Furanen und PCB in Baden-Württemberg. Abschlußbericht zum Forschungsvorhaben Nr. U 44–90.09 1995. Umweltministerium Baden-Württemberg, Stuttgart.

Hanf V, Brunner H, Haimovici F, Tinneberg HR, Hagenmaier H, Anderson D (1992) Influence of various dioxins on *in vitro* motility of human sperm. Chemosphere 25:1049–1052.

Hanf V, Behnisch P, Körner W, Sonntag CP, Tinneberg HR, Hagenmaier H (1995) Concentrations and congener profiles of PCB in human preovulatory cervical mucus and effects on sperm motility *in vitro*. Organohalogen Compounds 26:155–159.

Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EHH, Horte A, Jensen TK, Jouannet P, Keiding N, Vierula M, Toppari J, Skakkebaek NE (2001) Regional differences in semen quality in Europe. Hum Reprod 16:1012–1019.

Jackson MB (1988) The epidemiology of cryptorchism. Horm Res 30:153–156.

de Jager C, Bornman MS, van der Horst G (1999a) 1. The effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. Andrologia 31, 99–106.

de Jager C, Bornman MS, Oosthuizen J MC (1999b) 2. The effect of p-nonylphenol on fertility potential in male rats after gestational, lactational and direct exposure. Andrologia 31:107–113.

de Jager C, Bornman MS, Wandrag S, Sharp VW (2001) The lethal dose and potential reproductive effects of p-nonylphenol in rats: a preliminary study. Arch Androl 46:183–187.

Jobling S, Reynolds T, White JA, Parker MG, Sumpter JP (1995) A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582–587.

Jung A, Eberl M, Schill W-B (2001) Improvement of semen quality by noctural scrotal cooling and moderate behavioural change to reduce genital heat stress in men with oligoteratozoospermia. Reproduction 121:595–603.

Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T, Tilson HA (1996) Research needs for risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ Health Perspect 104:715–740.

Kholkute SD, Rodriguez J, Dukelow WR (1994a) Effects of polychlorinated biphenyls (PCBs) on *in vitro* fertilization in the mouse. Reprod Toxicol 8:69–73.

Kholkute SD, Rodriguez J, Dukelow WR (1994b) Reproductive toxicity of aroclor 1254: effects on oocyte, spermatozoa, *in vitro* fertilization, and embryo development in the mouse. Reprod Toxicol 8:487–493.

Kim IS (2001) Effects of exposure of lactating female rats to polychlorinated biphenyls on testis weight, sperm production and Sertoli cell numbers in the adult male offspring. J Vet Med Sci 63:5–9.

Kimbrough RD (1995) Polychlorinated biphenyls (PCB) and human health: an update. Crit Rev Toxicol 25:133–163.

Kyselova V, Peknicova J, Buckiova D, Boubelik M (2003) Effects of p-nonylphenol and reseratrol on body and organ weight and *in vivo* fertility of outbred CD-1 mice. Reprod Biol Endocrinol 30:1–10.

Lamb JC, Moore JA (1981) Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 8:835–844.

Larsen SB, Abell A, Bonde JP (1998) Selection bias in occupational sperm studies. Am J Epidemiol 147:681–685.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggirei F, Mazzeo P (2003) *In utero* exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 111:1783–1785.

Laue L, Chan WY, Hsueh AJ, Kudo M, Hsu SY, Wu SM, Blomberg L, Cutler GB Jr (1995) Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. Proc Natl Acad Sci USA 92:1906–1910.

Lee PC (1998) Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats. Endocrine 9:105–111.

Lerchl A, Nieschlag E (1996) Gibt es eine Spermienkrise? Dt Ärztebl 93:A2465–A2468.

Li LH, Jester WF, Orth JM (1998) Effects of relatively low levels of mono(2-ethylhexyl)phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. Toxicol Appl Pharmacol 153:258–265.

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. Effects on spermatogenesis and reproductive capability. Toxicol Appl Pharmacol 114:118–126.

Marshburn PB, Sloan CS, Hammond MG (1989) Semen quality and association with coffee drinking, cigarette smoking, and ethanol consumption. Fertil Steril 52:162–165.

Martin MM, Wu SM, Martin AL, Rennert OM, Chan WY (1998) Testicular seminoma in a patient with a constitutively activating mutation of the luteinizing hormone/chorionic gonadotropin receptor. Eur J Endocrinol 139:101–106.

Matthews JB, Twomey K, Zacharewski TR (2001) *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors α and β. Chem Res Toxicol 14:149–157.

Mettang T, Thomas S, Kiefer T, Fischer F-P, Kuhlmann U, Wodarz R, Rettenmeier AW (1996) The fate of leached di(2-ethylhexyl)phthalate (DEHP) in patients undergoing CAPD treatment. Perit Dial Int 16:1–4.

Nagao T, Saito Y, Usumi K, Kuwagata M, Imai K (1999) Reproductive function in rats exposed neonatally to bisphenol A and estradiol benzoate. Reprod Toxicol 13:303–311.

Odum J, Ashby J (2000) Neonatal exposure of male rats to nonylphenol has no effect on the reproductive tract. Toxicol Sci 56:400–404.

Odum J, Lefevre PA, Tittensor S, Paton D, Routledge EJ, Beresford NA, Sumpter JP, Ashby J (1997) The rodent-uterotrophic assay: critical protocol features, studies with nonylphenols, and comparison with a yeast estrogenicity assay. Regul Toxicol Pharmacol 25:176–188.

Osser S, Beckman-Ramirez A, Liedholm P (1992) Semen quality of smoking and non-smoking men in infertile couples in a Swedish population. Acta Obstet Gynecol Scand 71:215–218.

Osterlind A (1986) Diverging trends in incidence and mortality of testicular cancer in Denmark, 1943–1982. Br J Cancer 53:501–505.

Pflieger-Bruss S, Hagemann S, Heitkamp S, Köhn FM, Körner W, Hanf V, Schill WB (1999a) Effects of chlorinated hydrocarbons on the acrosome reaction of human spermatozoa *in vitro*. Hum Reprod 14:14.

Pflieger-Bruss S, Hanf V, Behnisch P, Hagenmaier H, Rune GM (1999b) Effects of single polychlorinated biphenyls on the morphology of cultured rat tubuli seminiferi. Andrologia 31:77–82.

Pollack GM, Buchanan JF, Slaughter RL, Kohli RK, Shen DD (1985) Circulating concentrations of di(2-ethylhexyl)phthalate and its de-esterified phthalic acid products following plasticizer exposure in patients receiving hemodialysis. Toxicol Appl Pharmacol 79:257–267.

Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I (1997) Subchronic oral toxicity of di-n-octyl phthalate and di(2-ethylhexyl)phthalate in the rat. Food Chem Toxicol 35:225–239.

Pottenger LH, Domoradzki JY, Markham DA, Hansen S, Cagen, SZ, Waechter JM (2000) The relative bioavailability and metabolism of bisphenol A in rats dependent upon the route of administration. Toxicol Sci 54:3–18.

Raychoudhury SS, Flowers AF, Millette CF, Finlay MF (2000) Toxic effects of polychlorinated biphenyls on cultured rat Sertoli cells. J Androl 21:964–973.

Roediger B, van der Ven H, Schlebusch H, Wagner U, Knapp M, Al-Hasani S, Dietrich K, Krebs D (1989) Einfluß von Pestiziden auf die Funktion von Spermatozoen *in vitro*. Arch Gynecol Obstet 245:1041–1042.

Routledge EJ, Sumpter JP (1997) Structural features of alkylphenolic chemicals associated with estrogenic activity. J Biol Chem 272:3280–3288.

Rowley MJ, Leach DR, Warner GA, Heller CG (1974) Effects of graded doses of ionizing radiation on the human testis. Radiat Res 59:665–678.

Rozati R, Reddy PP, Reddanna P, Mujtaba R (2002) Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril 78:1187–1194.

Rune GM, DeSouza P, Krowke R, Merker HJ, Neubert D (1991a) Morphological and histochemical pattern of response in rat testes after administration of 2,3,7,8-tetracholorodibenzo-p-dioxin (TCDD). Histol Histopathol 6:459–467.

Rune GM, DeSouza P, Krowke R, Merker HJ, Neubert D (1991b) Morphological and histochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on marmoset (*Callithrix jacchus*) testes. Arch Androl 26:143–154.

Sakaue M, Ohsako S, Ishimura R, Kurosawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C (2001) Bisphenol A affects spermatogenesis in the adult rat even at a low dose. J Occupational Health Psychol 43:185–190.

Schäfer WR, Zahradnik HP, Frijus-Plessen N, Schneider K (1996) Anthropogene Substanzen mit unerwünschter Östrogenwirkung. Umweltmedizin 1:35–42.

Schlebusch H, Wagner U, van der Ven H, Al-Hasani S, Dietrich K, Krebs D (1989) Polychlorinated biphenyls: the occurrence of the main congeners in follicular and sperm fluids. J Clin Chem Clin Biochem 27:663–667.

Schönfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I (2002) Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect 110:A703–A707.

Setchell BP (1997) Sperm counts in semen of farm animals 1932–1995. Int J Androl 20:209–214.

Sharpe RM (1995) Another DDT connection. Nature 375:538–539.

Skakkebaek NE, Rajpert-De Meyts E, Main KM (2001) Testicular dysgenesis syndrome: an increasingly common development disorder with environmental aspects. Hum Reprod 16:972–978.

Soto AM, Justicia H, Wray WW, Sonnenschein C (1991) p-Nonyl-phenol: an estrogenic xenobiotic released from 'modified' polystyrene. Environ Health Perspect 92:167–173.

Surber C, Rufli T (1995) Lindan. Hautarzt 46:528–536.

Thomas JA, Thomas MJ (1984) Biological effects of di-(2-ethylhexyl)phthalate and other phthalatic acid esters. Crit Rev Toxicol 13:283–317.

Tielemans E, van Kooij R, te Velde ER, Burdoef A, Heederik D (1999) Pesticide exposure and decreased fertilisation rates *in vitro*. Lancet 354:484–485.

Villalobos M, Olea N, Brotons JA, Olea-Serrano MF, Ruiz de Almodovar JM, Pedraza V (1995) The E-Sceen assay: a comparison of different MCF7 cell stocks. Environ Health Perspect 103:844–850.

Völkel W, Colno T, Csanady GA, Filser J, Dekant W (2002) Metabolism and kinetics of bisphenol A in human at low doses following oral administration. Chem Res Toxicol 15:1281–1287.

Wagner U, Schlebusch H, van der Ven H, Dietrich K, Krebs D (1990) Accumulation of pollutants in the genital tract of sterility patients. J Clin Chem Clin Biochem 28:683–688.

Wine RN, Li LH, Hommel Barnes L (1997) Reproductive toxicology of di-n-butylphthalate in a continuous breeding protocol in Sprague-Dawley rats. Environ Health Perspect 105:102–107.

Wong JS, Gill SS (2002) Gene expression changes induced in mouse liver by di(2-ethylhexyl)phthalate. Toxicol Appl Pharmacol 185:160–196.

World Health Organization (2000) WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge University Press, Cambridge, UK.

Zavos PM, Correa JR, Karagounis CS, Ahparaki A, Phoroglou C, Hicks CL, Zarmakoupis-Zavos PN (1998) An electron microscope study of the axonemal ultrastructure in human spermatozoa from male smokers and nonsmokers. Fertil Steril 69:430–434.