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Effects of chlorinated hydrocarbons on sperm function *in vitro*

S. Pflieger-Bruss and W.-B. Schill

Centre of Dermatology and Andrology, Justus Liebig University, Giessen, Germany

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Summary. For the past few years there has been controversial discussion of whether environmental pollutants in general, and chlorinated hydrocarbons in particular, may impair male fertility. Organochlorine compounds, e.g. dichlorodiphenyltrichlorothane (DDT) and metabolites, polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are highly persistent in the environment and there is therefore some concern about human exposure. These chlorinated compounds are universally found in human body fluids. Substantial amounts are detected in human body fluids associated with reproduction such as follicular fluid, seminal fluid and cervical mucus. The available data on male fertility and organochlorines are scarce and controversial. Fertilization rates in *in vitro* fertilization procedures were found to be decreased in couples in whom the male partner was exposed to pesticides. Data on the effects of PCBs on human sperm motility and acrosome reaction *in vitro* are controversial. Various PCDD congeners had no effect on human sperm motility *in vitro*. Effects of chlorinated hydrocarbons on sperm function *in vivo* seem to be unlikely since the concentrations used *in vitro* were far higher than those found in fluids of the human reproductive tract. However, negative effects on human spermatozoa *in vivo* cannot be totally excluded because other organohalogen compounds can be identified in the genital tract and little is known about their synergistic effects.

Introduction

The report by Carlsen *et al.* (1992), in which it was suggested that mean sperm counts in men have declined in the past 50 years, started a controversial discussion on whether environmental pollutants in general and chlorinated hydrocarbons in particular may impair male fertility. The organochlorine compounds comprise dichlorodiphenyltrichloroethane (DDT) and metabolites gamma-hexachlorocyclohexane (gamma-HCH), polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs). Except for gamma-HCH (lindane) the production and application of these chemicals has been banned in Germany. Nevertheless, there is ongoing exposure because of their high persistence in the environment and the import of contaminated products, especially from eastern countries. Due to their lipophilicity they preferentially bio-accumulate 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.

Tris(4-chlorophenyl)methanol (TCPM), another organochlorine compound, has been detected as a contaminant in the marine environment since 1989 (Walker *et al.*, 1989). In recent years it has been found in various parts of the world. It is therefore another ubiquitous pollutant which is significantly correlated with the presence of most other high molecular weight organochlorines (Jarman *et al.*, 1992). To date, very little is known about its production and application, and there is a lack of knowledge of reproductive toxicology.

The regulation of new chemicals is mainly based on animal models, and data on human reproductive toxicity continue to be sparse and limited.

Correspondence: Dr S. Pflieger-Bruss, Centre of Dermatology and Andrology, Justus Liebig University, Gaffkystrasse 14, 35385 Giessen, Germany. Tel.: +49–641–99–43220; Fax: +49–641–99–43259.

Human exposure to chlorinated hydrocarbons

Although the production and application of DDT, PCBs and related compounds have been banned in most industrialized countries, they are still universally found in biological samples. Mexico, for instance, has used 3000 tons of DDT per year in its anti-malaria control programmes, mainly in tropical areas, since 1990 (Pardio *et al.*, 1997). Therefore, DDT concentrations are found in high amounts in mother's milk and human tissues. In Europe the exposure risk mainly consists of the import and consumption of contaminated products.

The PCB concentrations in biological samples have decreased worldwide in the past 20 years (Kimbrough, 1995). Nevertheless there is still concern about human exposure due to the presence of PCBs in capacitors and transformers that are still in operation. Additionally, PCBs are formed *de novo* through incineration (Hagenmaier *et al.*, 1995). PCBs are polycyclic polychlorinated aromatic hydrocarbons which are chemically closely related to PCDDs/PCDFs, although they are thought to be less toxic (Ahlborg *et al.*, 1992). A total of 209 PCB congeners can be distinguished, varying in the number and position of chlorines in the molecule. However, in the environment 132 PCB congeners have been found of different biological and toxicological relevance. Since PCBs are always present as complex mixtures in biological matrices, human risk assessment is very difficult. The total PCB concentration in human whole serum ranges between 2 and 7 $\mu g\,l^{-1}$ (Kimbrough, 1995).

The main exposure route for DDT, PCBs and related compounds is the consumption of meat, milk and dairy products. In 1987 the mean daily intake in industrialized countries was 4.9–8.7 μg PCB per person per day (Kimbrough, 1995). For infants breast feeding is the main exposure route, although levels of PCBs and PCDDs/PCDFs in human milk have decreased over the past few years. The median PCB levels in milk from mothers living in northern Germany have decreased by 60% in the past 12 years to a median concentration of 0.502 $mg\,kg^{-1}$ on a fat basis (Schade & Heinzow, 1998). In 1988 the mean daily intake of a breast-fed infant was 41 μg PCB per 3.5 kg body weight per day. Intake via inhalation and dermal exposure can be neglected for most individuals.

For gamma-HCH dermal exposure is of some relevance because it is used in the therapy of ectoparasitic diseases. Ectoparasitic diseases are increasingly diagnosed in all classes of society in Europe and in the US (Surber & Rufli, 1995). 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 various galenic formulations and in various concentrations. In Europe a 0.3% lindane formulation is used whereas in the US a 1% lindane preparation is taken; 0.3% lindane is considered to be sufficient to treat ectoparasitosis. According to the World Health Organization the accepted daily intake for lindane is 0.01 mg per kg per day (Surber & Rufli, 1995). During the 1980s 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).

For TCPM data on human exposure are very rare. There is a study reporting TCPM concentrations of 1.6–2.5 $\mu g\,kg^{-1}$ in Italian human milk (Rahman *et al.*, 1993). TCPM levels of up to several $mg\,kg^{-1}$ on a lipid weight basis were found in liver and adipose tissue of marine mammals, birds and fish from all over the world.

Some aspects of toxicology and reproductive toxicology of chlorinated hydrocarbons

PCBs and PCDDs/PCDFs exhibit a great variety of toxicological effects such as immuno-, hepato- and neurotoxicity, carcinogenesis, dermal lesions, induction of xenobiotic-metabolizing enzymes (e.g. cytochrome P450 isozymes) and reproductive effects. Some of these compounds show endocrine activities. While PCDDs and some PCB congeners (dioxin-like PCBs) are anti-oestrogens, others are weakly oestrogenic in action. Especially non-coplanar PCBs (mono- and di-ortho-substituted congeners) and their hydroxy-metabolites display oestrogenic activity in a number of test systems *in vitro* and *in vivo*.

Reproductive toxicology has been investigated in a number of animal studies. After exposure to high concentrations of PCBs, decreased conception rates (monkey, pig, mouse) (Allen *et al.*, 1974; Earl *et al.*, 1974; Merson & Kirkpatrick, 1976) increased abortion rates (monkey, rabbit, guinea pig) (Ahlborg *et al.*, 1992) and effects on the oestrous cycle (rat, mouse, monkey) (Örberg & Kihlström, 1973; Müller *et al.*, 1978; Brezner *et al.*, 1984) were found. Alterations of the cortisol, progesterone, oestrogen and testosterone levels were reported (Ahlborg *et al.*, 1992).

Male fertility and chlorinated hydrocarbons

Data on chlorinated hydrocarbons and human male fertility are scarce or controversial. Most data available are from animal models.

The effects of PCBs on male reproduction *in vivo* comprise reduced fertility in postnatally exposed rats, reduced matings (rat), decreased concentration of testicular spermatozoa (mouse), and reduced weight of the ventral prostate (rat) and seminal vesicles (rat, mouse) (Ahlborg *et al.*, 1992). Single nonortho and mono-ortho PCB induce morphological alterations in cultured rat tubuli seminiferi in a time- and stage-dependent manner (Pflieger-Bruss *et al.*, 1999a).

It has been shown that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) affects male fertility in the rat and the marmoset monkey (*Callithrix jacchus*) after high-dose exposure. Testicular morphology of marmosets (*Callithrix jacchus*) and rats was affected in a dose-dependent manner as revealed by light and electron microscopy (Rune *et al.*, 1991a,b). Additionally, a reduction of 3β- and 17β-hydroxysteroid-dehydrogenase activity in rat Leydig cells was found by histochemical procedures (Rune *et al.*, 1991a). In male mice exposed for 8 weeks to high doses of 2,3,7,8-TCDD, no effects on fertility were found and no abnormalities were detected in their offspring (Lamb & Moore, 1981). While *in utero* and lactational TCDD exposure reduced sperm production in male rats, fertility was not affected (Mably *et al.*, 1992). This is not contradictory because rats produce far more spermatozoa than are necessary for their unrestricted fertility.

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

In humans a correlation between standard semen parameters (motility, morphology and sperm count) and the concentration of PCB and gamma-HCH in the seminal plasma was not found (Ensslen *et al.*, 1990).

Sperm function and chlorinated hydrocarbons

Sperm functions that are necessary for normal fertilizing capacity of spermatozoa comprise motility, vitality, acrosome reaction, penetration through the cumulus, binding to the zona pellucida and subsequently fusion with the oocyte membrane. In mammals and humans, spermatozoa must undergo a number of changes before they gain their fertilizing ability. During their passage through the female reproductive tract several biochemical changes occur in their plasma membranes, which enable them to undergo the acrosome reaction. Prematurely acrosome-reacted spermatozoa lose their fertilizing potential (Cherr *et al.*, 1986).

It was shown that DDT and metabolites, gamma-HCH and different PCB congeners are present in the follicular, seminal and cervical fluid (Schlebusch *et al.*, 1989; Wagner *et al.*, 1990; Hanf *et al.*, 1995). Tielemans *et al.* (1999) suggested that pesticide exposure decreases human sperm fertilizing ability *in vitro*. Fertilization rates in the *in vitro* fertilization (IVF) treatment were significantly lower for couples with male partners occupationally exposed to pesticides (Tielemans *et al.*, 1999). However, the authors did not draw conclusions about which chemical may be responsible for that effect. Kholkute *et al.* (1994a) reported that the commercial PCB mixtures Aroclor 1221, 1254, 1268 and 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) reduce fertilization rates in IVF procedures in mice. Mouse spermatozoa were capacitated in PCB-free medium, whereas oocytes were cultured in medium containing PCB (Kholkute *et al.*, 1994a). Capacitation of mouse spermatozoa in Aroclor-1254-containing medium, followed by co-culture with untreated oocytes, failed to affect the IVF rate (Kholkute *et al.*, 1994b). Mouse sperm motility was unaffected after exposure to 1 and 10 $\mu g\ ml^{-1}$ of Aroclor 1254. No effect of 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) on the motility of human spermatozoa *in vitro* was found (Hanf *et al.*, 1995). The PCB 77 concentration in the sperm cells after completion of the experiment was 421.4 $\mu g\ g^{-1}$ fat and therefore bioavailable (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 *in vitro*. The 2,3,7,8-TCDD concentration in the medium (0.72 $ng\ ml^{-1}$) was about 10 000-fold above the average concentration in body fluids. In contrast, Roediger *et al.* (1989) demonstrated that human sperm motility decreased compared with control after exposure to 2,2′,6,6′-tetrachlorobiphenyl. These effects were found at very low PCB concentrations (1–10 $ng\ ml^{-1}$). Additionally, induction of the acrosome reaction was reported (Roediger *et al.*, 1989). Data from our own laboratory suggest that very high concentrations (2.5–20 $\mu g\ ml^{-1}$) of PCB 77, 126, 153 and 118 do not affect motility and vitality of human spermatozoa *in vitro* (Pflieger-Bruss *et al.*, 1999b). 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.*, 1999b). TCPM affected sperm vitality and motility in a dose-dependent manner. Effects were obvious after high-dose exposure. The *in vitro* effects of chlorinated hydrocarbons were observed at concentrations far higher than those found in fluids of the human reproductive tract. Nevertheless, negative effects on sperm function cannot be totally excluded as other organohalogen compounds can be identified in the genital tract and their synergistic effects are mostly unknown.

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