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Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits

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The female rabbit was used to study (i) accumulation of lipophilic chlorinated hydrocarbons in genital tract tissues and (ii) subsequent morphological and functional effects after long-term low-dose exposure. Polychlorinated biphenyl (PCB), 1,1-di(p-chlorophenyl)-2,2,2-trichloroethane (DDT) and γ-hexachlorocyclohexane (γ-HCH) (dosages: 4, 3 and 0.8 mg per kg body weight, respectively) and a combination of these three components (and dosages) were administered to sexually mature rabbits over a period of 12–15 weeks. The animals were killed shortly before and at various times after ovulation. Accumulation of chlorinated hydrocarbons was high in ovarian, oviductal and uterine tissues, in follicular fluid and clearly detectable in uterine secretions. In follicular fluid, the concentration and patterns of congeners and isomers of PCB and DDT were distinctly different from serum. DDT- and γ-HCH-treated animals showed a significantly reduced ovulation rate ($P < 0.002$ and 0.05, respectively). During early pregnancy DDT decreased serum progesterone levels and changed the protein pattern of uterine secretion. Functional effects, however, were much less expressed compared with the highly significant accumulation of the persistent organochlorines in the genital tract.

Key words: chlorinated hydrocarbons/female rabbit/impaired ovulation/preimplantation period/tissue accumulation

Introduction

Chlorinated hydrocarbons have been widely used as insecticides or industrial compounds for several decades. Their accumulation in the environment was first described in the 1960s (Risebrough *et al.*, 1968). Although their application has been restricted in most industrialized countries, they are still prevalent due to their chemical stability and low degradation. Traces of organochlorines were even found in the Arctic region (WHO, 1979; Oehme, 1991). Their lipophilic character leads to preferential uptake by and high accumulation in fatty tissues and breast milk (Deutsche Forschungsgemeinschaft, 1988; Deutsche Gesellschaft für Ernährung, 1988). Recently, chlorinated hydrocarbons were even detected in follicular fluid of women not being specifically exposed to these substances (Baukloh *et al.*, 1985; Schlebusch *et al.*, 1989).

Some of the environmental pollutants exhibit endocrine actions (Bitman *et al.*, 1968; Gellert *et al.*, 1972; Kupfer and Bulger, 1982) and may therefore contribute to fertility disorders in animals and the human. In many cases, the causes for infertility are unknown (Beier, 1988; Page, 1989). Systematic studies of possible relations between the accumulation of lipophilic pollutants in tissues and fluids of the genital tract and the incidence of reproductive disorders are lacking. In particular, the preimplantation period of pregnancy has been widely ignored so far.

We investigated the accumulation of orally administered polychlorinated biphenyl (PCB), 1,1-di(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) and γ-hexachlorocyclohexane (γ-HCH) in tissues and fluids of the genital tract of female rabbits and their effect on reproductive functions. To mimick potential exposure to organochlorines as realistically as possible, the xenobiotics were given in a low dose over a long period (12–15 weeks), either individually or as a mixture of the three compounds investigated.

Materials and methods

Organochlorine compounds

PCB: Aroclor 1260, a technical PCB mixture containing 60% fixed chloride (producer: Monsanto, St Louis, MO, USA). DDT: a technical mixture of ~15–20% *op*- and 80–85% *pp*-isomers (producer: Dr Ehrenstorfer, Augsburg, Germany). γ-HCH: γ-isomer of HCH with a purity of 99.1% (producer: Dr Ehrenstorfer, Augsburg, Germany). All compounds were purchased from Promochem, Wesel, Germany.

The organochlorine compounds were dissolved in 6 ml corn oil to achieve a standard dosage of 4 mg/kg body weight (BW) (PCB), 3 mg/kg BW (DDT) and 0.8 mg/kg BW (γ-HCH), respectively. The dosage of each substance has been determined in preliminary studies involving different doses of PCB, DDT and γ-HCH (Lindenau, 1992; Seiler, 1993) and according to literature data (Villeneuve *et al.*, 1971; Hart *et al.*, 1972; Sircar and Lahiri, 1989), ensuring a non-toxic dose for the treated animals.

Animals and treatments

Sexually mature female hybrid rabbits (Fischer and Schumacher, 1991) with body weights ranging from 3 to 4.5 kg were used for this study. They were housed and cared for as previously

described (Fischer and Meuser-Odenkirchen, 1988). PCB, DDT, γ-HCH or a combination of all three compounds were administered via gavage three times per week for 12 or 13 (PCB only) weeks. After that time the females were artificially inseminated (Fischer and Meuser-Odenkirchen, 1988) and were killed by cervical dislocation and exsanguination 1, 6 or 11 days post-insemination. Organochlorine administration was continued until euthanasia. Control animals received 6 ml of the vehicle, i.e. of pure corn oil. Animals designed for the collection of follicular fluid (day 0) were treated with 0.3 mg follicle stimulating hormone (FSH; Schering Corp., Kenilworth, NJ, USA) in the morning and evening for three consecutive days (Fischer and Meuser-Odenkirchen, 1988). Ovulation was induced the next morning by i.v. injection of 75 IU human chorionic gonadotrophin (HCG, Prolan; Bayer Leverkusen, Germany). After euthanasia, the reproductive tract, liver and peri-renal fat were removed for further processing. The experimental groups and animal numbers are given in Table I.

Table I. Treatment groups and animals numbers

Treatment	Doses	Day 0[a]	Day 1	Day 6	Day 11
PCB	C: 6 ml corn oil		$n = 5$	$n = 5$	$n = 5$
	T: 4 mg/kg BW	$n = 5$	$n = 5$	$n = 5$	$n = 5$
DDT	C: 6 ml corn oil		$n = 5$	$n = 5$	$n = 5$
	T: 3 mg/kg BW	$n = 5$	$n = 5$	$n = 5$	$n = 5$
γ-HCH	C: 6 ml corn oil		$n = 5$	$n = 5$	$n = 5$
	T: 0.8 mg/kg BW	$n = 5$	$n = 5$	$n = 5$	$n = 5$
Combination	C: 6 ml corn oil		$n = 5$	$n = 5$	$n = 5$
	T: 4 mg PCB + 3 mg DDT + 0.8 mg γ-HCH/kg BW		$n = 6$	$n = 7$	$n = 6$

[a]Day 0 = day of insemination.
C = control; T = treatment; BW = body weight; n = number of animals.

Sample collection and preparation

Serum

Blood samples were drawn from the arteriae auriculares once a week for residue analyses and every second day from pregnant animals for hormone assays. Samples were centrifuged at 1000 *g* for 15 min.

Follicular fluid

About 9½ h after injection of HCG, i.e. shortly before ovulation, follicles of ≥ 1 mm in diameter were punctured. Contamination with blood or granulosa cells was carefully avoided.

Oviductal and uterine flushing

Oviducts and uteri were flushed twice. They were first flushed with warmed Ringer's solution to recover cleavage stages (day 1) or blastocysts (day 6) and for electrophoretic analysis of uterine secretory proteins. For better dissolution of the organochlorines, the second flushing was performed with a lipophilic additive, 2% tetramethylurea (TMU). These flushings were used for residue analyses.

Ovary

After counting the ovulation points (day 1 post-insemination) and corpora lutea (day 6 and 11 post-insemination) fatty tissue was carefully removed and the ovaries were frozen until residue analyses. In the DDT experiment, corpora lutea were enucleated from the stroma for separate residue analyses. For histological evaluation and for measurement of a prostaglandin $PGF_{2\alpha}$ metabolite, ovaries were fixed with Bouin's solution or frozen in liquid nitrogen, respectively.

Oviduct and uterus

For residue analysis tissues were prepared free of fat. Representative pieces from the middle of the uterus were cut and fixed in Bouin's solution for histological evaluation.

All specimens were weighed before storage at −20°C.

Radioimmunoassays for oestradiol, progesterone and prostaglandin $F_{2\alpha}$ metabolite

Concentrations of serum oestradiol and progesterone were measured using commercial radioimmunoassay kits purchased from Biermann GmbH (oestradiol; Bad Nauheim, Germany) and Amersham Buchler (progesterone; Braunschweig, Germany). The sensitivity of the assay was 0.25 nmol/l and 25.4 pmol/l, respectively. Concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were measured by a radioimmunoassay as described by Schlegel *et al.* (1982).

Histology

Bouin-fixed samples of the uterus and ovary were embedded in paraffin and dehydrated by routine methods. Sections of ~5 μm were cut and stained with haematoxylin and eosin.

One-dimensional SDS–PAGE gels

Protein concentration of the uterine flushing was determined according to Lowry *et al.* (1951). The protein composition was analysed by one-dimensional denaturating sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970), using a 8.3–16% acrylamide separating gel. The stacking gel contained 5% acrylamide. Each lane was loaded with 50 μg protein diluted with sample buffer. The gels were stained with Coomassie brilliant blue. Quantitative evaluation was performed with a laser densitometer (Ultroscan XL, LKB Bromma, Sweden) as described before (Beier-Hellwig *et al.*, 1988).

Residue analyses

The concentration of chlorinated hydrocarbons in fluids and tissues was determined gas-chromatographically following extraction with chloroform and petroleum benzine according to Stijve and Cardinale (1974). Standards and extraction efficiencies were as stated by Ruoff *et al.* (1988). The detection limit for PCB, DDT and γ-HCH was 0.0001 mg/kg wet weight. The accumulation of PCB is given as a sum of six indicator congeners (28, 52, 101, 153 and 180) according to the nomenclature of Ballschmiter and Zell (1980). DDT residues were measured as *op*- and *pp*-isomers.

Health status

The animals were under medical control during experiments. At the end of treatment blood samples were drawn from each rabbit

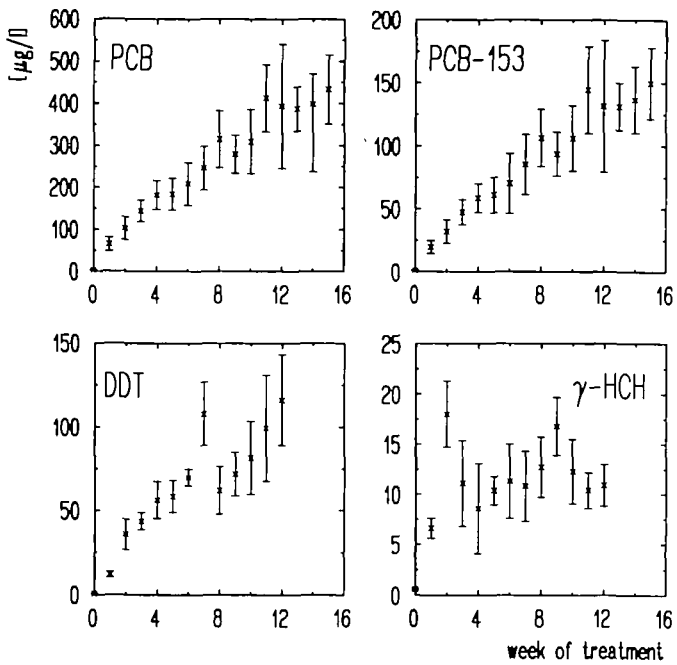

Fig. 1. Concentration of polychlorinated biphenyl (PCB), PCB congener 153, 1,1-di(*P*-chlorophenyl)-2,2,2-trichloroethane (DDT) and γ-hexachlorocyclohexane (γ-HCH) in serum after one compound exposure; mean ± SEM for $n = 5$ animals (PCB) and $n = 3$ animals each (DDT, γ-HCH).

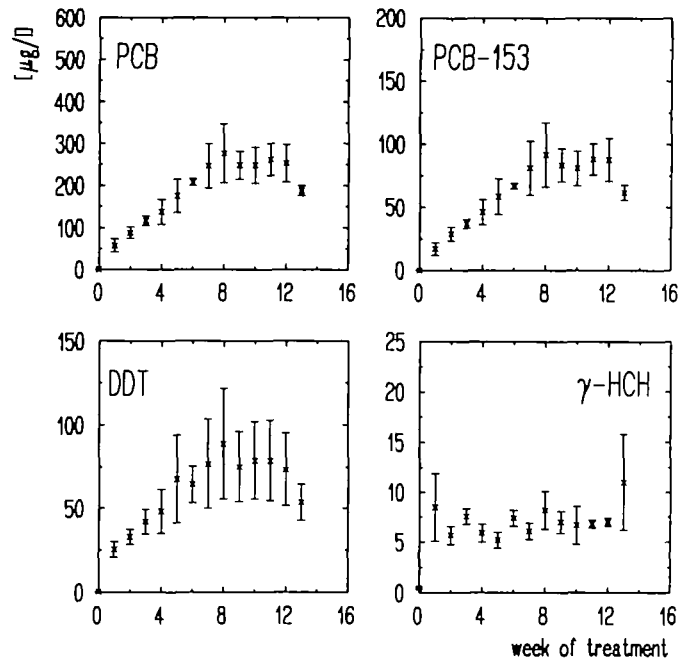

Fig. 2. Accumulation of PCB, PCB congener 153, DDT and γ-HCH in serum after combination treatment; mean ± SEM for $n = 3$ animals each. Abbreviations as in Figure 1.

to determine routine clinical–chemical and haematological blood and serum parameters.

Statistical analysis

Differences in ovulation numbers between treatment and control groups were assessed by the Wilcoxon matched-pairs signed-rank test. In all other cases Student's *t*-test was applied (Sachs, 1984).

Results

Animal health status

Clinical inspection and blood parameters did not indicate any sign of impaired health in the PCB-, DDT- and γ-HCH-treated animals. Thus, effects of a long-term exposure to chlorinated hydrocarbons rather than acute toxic effects could be investigated.

Residue analysis

Serum

Serum concentration of PCB, DDT and γ-HCH during the time of treatment is illustrated in Figure 1. In the one-compound treatments, the concentration of PCB and DDT increased until the end of the study, while γ-HCH residues showed two maxima. PCB congener 153 closely reflected the increase of total PCB (see also Figure 2). After combination treatment (Figure 2), the concentration of each compound amounted to only 60–70% of the single compound experiments and the maximal concentration was already achieved in the 9th week. Residues of DDT declined at the end of combination treatment.

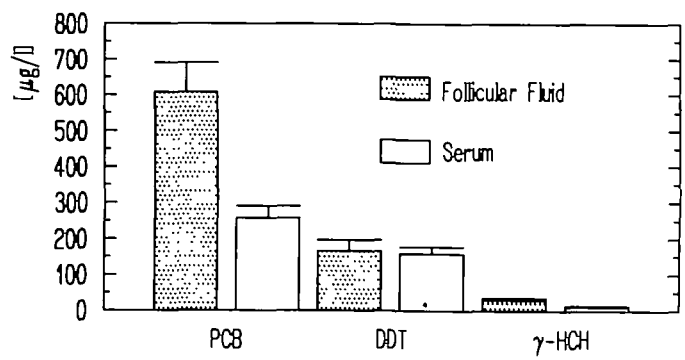

Fig. 3. Concentration of PCB, DDT and γ-HCH in follicular fluid and corresponding serum after one compound exposure (mean ± SEM, $n = 5$); $P < 0.01$ for PCB and γ-HCH. Abbreviations as in Figure 1.

Table II. Relative amounts (mean ± SEM for $n = 5$ animals) of the highly chlorinated PCB congeners 138, 153, 180 and the *op*- and *pp*-DDT isomers (in % of total concentration)[a]

	Serum	Follicular fluid
Congener 138	31.4 ± 3.9	28.3 ± 3.9
Congener 153	34.4 ± 4.8	32.6 ± 4.0
Congener 180	33.8 ± 3.8	38.4 ± 4.2
op-Isomer	0.5 ± 0.1	4.9 ± 0.9
pp-Isomer	99.4 ± 6.3	95.1 ± 8.8

[a]All comparisons $P > 0.05$.

Follicular fluid

The level of DDT in follicular fluid did not exceed that of serum. The concentration of PCB and γ-HCH, however, was 2.4- and

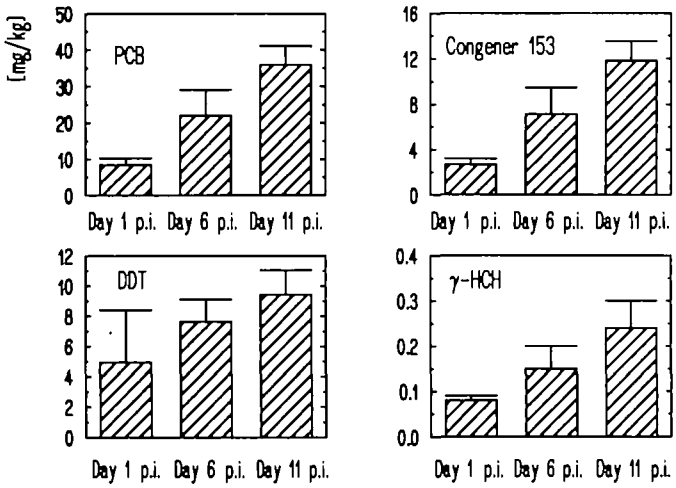

Fig. 4. Accumulation of PCB, PCB congener 153, DDT and γ-HCH in ovarian tissue from day 1 post-insemination (p.i.) to day 11 p.i. after one compound exposure; (mean ± SE, n = 5 animals). Abbreviations as in Figure 1.

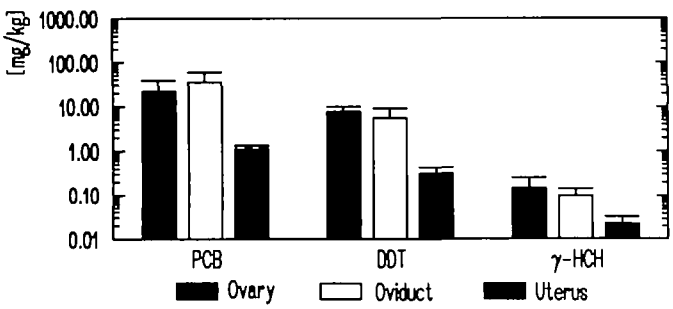

Fig. 5. Accumulation of PCB, DDT and γ-HCH in ovarian, oviductal and uterine tissue after one-compound treatments. Comparison based on corresponding tissues derived from the same animals in each case (day 6 post-insemination; mean ± SEM, n = 5 animals); ovary versus oviduct: $P > 0.05$; ovary versus uterus: $P < 0.01$; oviduct versus uterus: $P < 0.01$ for each substance. Abbreviations as in Figure 1.

2.6-fold higher, respectively, than in the corresponding autologous serum (Figure 3). The distribution of specific PCB-congeners and DDT isomers (Table II) showed the preferential accumulation of congener 180 and *op*-DDT in follicular fluid. The highly persistent congener 180 is known to be a strong inductor of microsomal enzymes (Parkinson and Safe, 1987) and *op*-DDT (Bitman *et al.*, 1968; Nelson, 1974; Stancel *et al.*, 1980) is the strongest endocrine-effective isomer of DDT.

Ovary, oviduct and uterus

The accumulation in the ovary at day 1, day 6 and day 11 reflects the high affinity of the organochlorine compounds for lipophilic tissue. Concentrations of PCB, DDT and γ-HCH increased in parallel with advancing luteinization (Figure 4). In the DDT experiment, corpora lutea from day 6 ovaries were enucleated and separately analysed. The mean levels of DDT in the corpora lutea were 8.2 compared with 2.8 mg/kg wet weight in the ovarian stroma. Comparable high accumulations as in day 6 ovaries were found in oviductal tissue, while uterine tissue

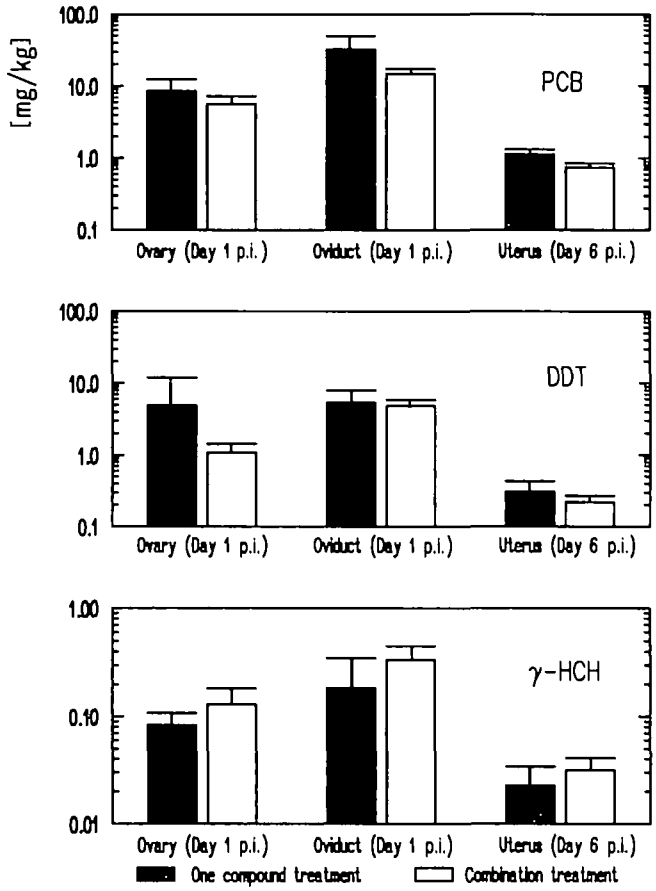

Fig. 6. Accumulation in the ovary, oviduct and uterus after one-compound treatment and combination treatment (mean ± SEM, n = 5 and n = 3 animals, respectively); $P < 0.05$ for the oviduct and $P < 0.01$ for the uterus in the PCB experiment, in all other cases $P > 0.05$. p.i. = post-insemination; other abbreviations as in Figure 1.

contained lower concentrations (Figure 5). This preference of organochlorines for the oviductal tissue was unexpected, as a lipid extraction revealed a lower lipid concentration in oviductal tissue compared with ovarian tissue (2.4 versus 8.2% lipid/kg wet weight, respectively). The low accumulations in uterine tissue (endometrium plus myometrium) correlated well with its low lipid content (0.6%). A comparison of accumulations in the one-compound experiments with the combined treatment (Figure 6) showed that PCB and DDT concentrations in genital tract tissues were lower after simultaneous administration of all three compounds, although γ-HCH concentration was higher. γ-HCH obviously has different physicochemical and metabolic characteristics than the other compounds, for example lower lipid solubility, shorter half-life and/or higher rates of excretion.

Oviductal and uterine flushing

Despite very high concentrations of PCB, DDT and γ-HCH in oviductal tissues (Figure 6), the organochlorines could not be detected in oviductal flushings (Table III). In uterine flushings notable concentrations of PCB and DDT and traces of γ-HCH were found. Again, the accumulation was higher in one-compound experiments than in a combination (Table III).

Table III. Accumulation of PCB, PCB congener 153, DDT and γ-HCH in oviductal (day 1 post-insemination) and uterine (day 6 post-insemination) flushing (ng/ml). Mean of $n = 5$ animals

	PCB	DDT	γ-HCH
OF single, combination	–	–	–
UF single	41.0	6.1	1.5
UF combination	11.6	2.7	–

'–' indicates no differences between exposed and control animals.
OF = oviductal flushing; UF = uterine flushing.

Table IV. Mean ovulation numbers after exposure to PCB, DDT and γ-HCH (combined data from day 1, 6 and 11 post-insemination)

	Control	Treated	
PCB	10.1 ± 1.6	11.2 ± 2.8	NS
DDT	11.9 ± 2.7	9.5 ± 1.3	$P < 0.002$
γ-HCH	11.8 ± 2.0	10.3 ± 1.3	$P < 0.05$
Combination	9.9 ± 1.3	9.3 ± 1.7	NS

Ovulation

The ovulation rate was significantly reduced after 12–15 weeks of DDT and γ-HCH treatment (Table IV). Surprisingly, a combined treatment, including DDT and γ-HCH in the same dose, as in the one-compound treatments, did not result in an impaired ovulation.

Histological evaluation

All stages of folliculogenesis, including atretic forms, were noticed in the ovarian cortex with no differences between treatment groups and between experimental and control females. Corpora lutea and lutein cells were regularly structured. No signs of degeneration, such as reduced size of lutein cells, pycnotic nuclei or fibroblast cell infiltration were found in corpora lutea of the chlorinated hydrocarbon-exposed rabbits.

The uterine morphology at day 1, scarcely different from a non-pregnant uterus, was characterized by a narrow radial lumen with low mucosal margin and an intact columnar cavum epithelium. At day 6, the luminal and glandular epithelium was highly proliferated with only a low portion of stroma, and uterine glands, symplasmata of the luminal epithelium, stromal vascularization and a thickened myometrium layer were dominating. No differences, however, could be demonstrated between uterine sections of control and exposed animals.

Electrophoresis of uterine flushing

The protein pattern of the uterine flushing was analysed at day 6 post-insemination, when uteroglobin, a progesterone-dependent protein secreted by the uterus of the pregnant rabbit (Beier, 1974, 1976, 1982), is by far the dominating protein. No differences concerning the total protein content as well as the electrophoretic pattern were found after exposure to PCB and γ-HCH. Only after DDT treatment did the relative proportion of uteroglobin decrease in favour of the other analysed protein fractions (Table V). This finding is in agreement with the drop in peripheral progesterone in DDT-treated animals (see Figure 7).

Table V. Relative amount (%) of four major SDS–PAGE protein fractions of uterine secretion after DDT-treatment[a]

	Uteroglobin	12.5 kDa	29 kDa	Albumin
Control				
Mean	69.8	6.5	3.5	2.8
SEM	3.4	0.9	0.5	0.9
Treated				
Mean	63.5	6.8	4.3	4.0
SEM	1.4	1.3	0.5	2.1

[a]Day 6 post-insemination, $n = 5$ animals, control versus treated: $P > 0.05$.

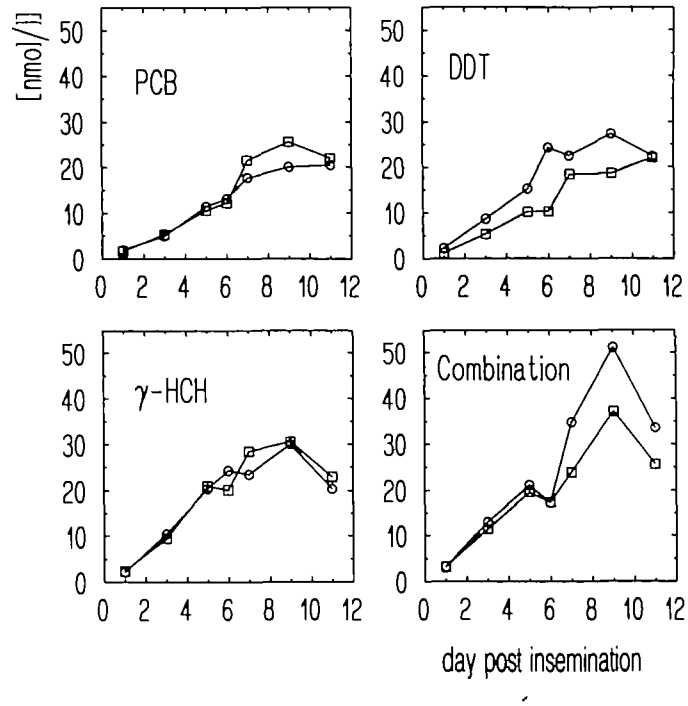

Fig. 7. Peripheral progesterone levels in early pregnancy in exposed (□) and control (○) females [day 1 post-insemination (p.i.) to day 11 p.i.]; means of $n = 10$ animals (until day 5 p.i.) and $n = 5$ animals from day 6 to day 11 p.i.; SEM ranged between 0.2 and 2.2 (PCB), 0.1 and 5.7 (DDT), 0.2 and 5.8 (γ-HCH) and 0.4 and 6.8 (combination); all comparisons: $P > 0.05$. Abbreviations as in Figure 1.

Oestradiol and progesterone

Peripheral serum oestradiol was characterized by substantial interindividual variation, a well-known phenomenon in the rabbit (Browning *et al.*, 1980; Orstead *et al.*, 1988). The environmental pollutants investigated did not affect mean oestradiol values in a specific manner. Likewise, progesterone levels were not influenced: a typical progestational increase was observed in all groups (Figure 7), except for DDT where a tendency towards lower serum progesterone was noticed ($P > 0.05$).

Prostaglandin $F_{2\alpha}$ metabolite

Based on studies of Lundkvist and Kindahl (1989) suggesting a PCB-induced increase in the synthesis of the luteolytic prosta-

glandin $F_{2\alpha}$, the concentration of prostaglandin $F_{2\alpha}$ metabolite was determined in peripheral serum and in the ovarian tissue (Schlegel *et al.*, 1988). There was no difference in the serum level between control and treated animals. The concentration was slightly higher in ovaries of PCB-exposed rabbits ($P > 0.05$; data not shown).

Discussion

The ubiquitous environmental pollutants PCB, DDT and γ-HCH accumulate in substantial concentrations in genital tract tissues and secretions after subchronic low-dose oral take up. DDT and γ-HCH exposure interfered with ovulation. DDT led to a decrease in peripheral progesterone and changed the uterine secretory protein pattern. Accumulation and effects of the organochlorines differed according to whether the compounds were administered individually or in combination.

All organochlorines were detectable in the serum almost immediately after the beginning of exposure (Figures 1 and 2). Serum concentrations of PCB and DDT increased until the end of single compound treatment, not reaching steady state. The different accumulation characteristics of γ-HCH in serum are due to the low level intake (0.8 mg/kg) and to its higher water solubility, leading to a shorter half-life. During and after combined treatment, accumulation of each substance was lower (Figures 1, 2, 5 and 6), reflecting a different kinetic. A tissue saturation of chlorinated hydrocarbons seemed to be reached; PCB levels plateaued and DDT residues even declined at the end of treatment. The intake of several organochlorine compounds obviously changed catabolism and excretion compared with a one-compound intake. Induction of microsomal enzymes by chlorinated hydrocarbons is supposed to be one of the involved metabolic mechanisms (Fahim *et al.*, 1970; Alvarez and Kappas, 1976; Safe, 1984; Macholz and Kujawa, 1985).

PCB and γ-HCH selectively accumulated in follicular fluid, the milieu directly surrounding the developing oocyte, as indicated by concentration and congener and isomer pattern (Table II). The composition of the fluid of tertiary follicles is similar to serum. But to some extent local secretions of granulosa cells contribute to its synthesis (Edwards, 1974; Gosden *et al.*, 1988). Our results indicate that lipids are not the only determinant for organochlorine accumulation since the lipid content of serum and follicular fluid are very similar (Edwards, 1974). Quantitative differences in protein pattern (Shalgi *et al.*, 1973; Engels, 1976) may be responsible for this, as some proteins, for instance albumin and especially lipoproteins, are known to bind hydrocarbons (Skalsky and Guthrie, 1978; Maliwal and Guthrie, 1982; Matthews *et al.*, 1984). Although fertilization was not impaired in any of the experimental groups (Seiler, 1993), the selective accumulation of a higher chlorinated and toxic PCB congener and a DDT isomer with oestrogenic properties in follicular fluid (Table II) is unphysiological and represents a potential danger. Comparable findings regarding absolute and specific accumulation of congeners and isomers have recently been reported for human specimens such as follicular fluid, cervical mucus and spermatozoa (Baukloh *et al.*, 1985; Schlebusch *et al.*, 1989; Wagner *et al.*, 1990; Ensslen *et al.*, 1990; Van der Ven *et al.*, 1991).

Developing corpora lutea are characterized by a high vascularization, an increase in size of luteal cells and storage of lipids (Hilliard *et al.*, 1968; Dharmarajan *et al.*, 1988, 1989). The storage of organochlorines and the great affinity of this ovarian compartment for chlorinated hydrocarbons (Figure 4) was proven by a differentiate determination of residues in corpora lutea and ovarian stromal tissue, with accumulations being high in corpora lutea and much lower in the ovarian stroma. The dramatic amounts of PCB, DDT and γ-HCH in oviductal tissue cannot be explained by its lipid content, as it is much lower compared with ovarian or uterine tissue. Further studies will have to identify the target tissue for organochlorines in the oviduct by analysing different compartments separately.

Accumulation of pollutants in oviductal and uterine secretions is of special significance because fertilization, early embryonic development, implantation, and fetal development could be affected. Flushing the oviduct and uterus with a lipophilic solvent resulted in clearly detectable accumulations in the uterine flushing at day 6, i.e. shortly before implantation takes place in the rabbit. Our findings are in accordance with earlier reports of DDT (Sieber and Fabro, 1971; McLachlan *et al.*, 1976) and PCB accumulation (Brandt *et al.*, 1982) in the uterine secretions. Recently, a PCB binding protein from rat clara cells and human broncho-alveolar cells has been identified and isolated (Lund *et al.*, 1985; Gillner *et al.*, 1988; Nordlund-Moller *et al.*, 1991; Buff and Bründl, 1992). It shows a high homology in the amino acid sequence with uteroglobin (Andersson *et al.*, 1991) which is the main protein in the rabbit progestational uterine secretion (Beier, 1968, 1974, 1976, 1982). Uteroglobin may be of specific importance for the distinct accumulation of the chlorinated hydrocarbons in the uterine flushing. Despite remarkably high concentrations of PCB, DDT and γ-HCH in oviductal tissue, no residues were found in the oviductal flushings. Oviduct secretions are characterized by low levels of uteroglobin during preimplantation period (Kay and Feigelson, 1972; Noske and Feigelson, 1976; Tucker and Schultz, 1977).

Concerning the influence of chlorinated hydrocarbons on reproductive functions, different experimental approaches showed that some of these compounds, especially DDT, exhibit endocrine properties (Peakall, 1967; Bitman *et al.*, 1978; Wrenn *et al.*, 1970; Kupfer, 1975). However, experimental results are not consistent and vary between species and compounds investigated, for example regarding degree of chlorination and the positions of chlorine in the molecule. For PCB, especially the lower chlorinated mixtures, and for DDT the *op*-isomers seem to interfere with physiological action of hormones (Bitman *et al.*, 1968; Gellert and Wilson, 1979; Galand *et al.*, 1987). Investigations in the rabbit are still lacking. A common in-vivo marker for oestrogenic activity in rats is a uterotropic response which was reported, after DDT or PCB administration, by Bitman *et al.* (1968), Gellert *et al.* (1972), Ecobichon and MacKenzie (1974) and McBlain *et al.* (1987). But in-vitro experiments, i.e. binding to uterine oestrogen receptor, also confirmed oestrogenic potencies of DDT and low chlorinated PCB mixtures (Nelson, 1974; Kupfer and Bulger, 1976). In the present investigation a higher chlorinated PCB mixture and a technical DDT with ~15–20% *op*-DDT have been used.

DDT and γ-HCH administered over a period of 12–15 weeks

caused significantly lower ovulation rates. We could not differentiate between the site of action, i.e. whether the effect was due to a direct endocrine effect on the ovary or on the hypothalamo-hypophysial axis. Ovaries and corpora lutea were morphologically intact. DDT-exposed animals showed lower levels of progesterone than the corresponding control during early pregnancy. This observation corresponds well with the decrease of uteroglobin in the uterine flushings of DDT-exposed females at day 6 post-insemination. Apart from direct effects of chlorinated hydrocarbons at the receptor, indirect effects via an induction of hepatic microsomal enzymes, especially of a P450-dependent mono-oxygenase, with possibly enhanced steroid metabolism (Fahim *et al.*, 1970; Villeneuve *et al.*, 1971; Bunyan *et al.*, 1972; Dwivedi and Kumar, 1989), may be responsible for these effects. Interestingly, both effects of DDT, the lower levels of serum progesterone and the reduced proportion of uteroglobin in uterine secretions, had no adverse biological consequences, as early embryonic development and implantation in DDT-exposed females were not affected (Seiler, 1993). With respect to the other chlorinated hydrocarbons studied, uterine transformation and secretions as well as steroid synthesis and prostaglandin $F_{2\alpha}$ metabolism seemed unaffected, despite clearly demonstrable accumulations. This discrepancy between accumulation of persistent organochlorines in some body tissues and fluids, and only small effects on reproductive organs, needs further investigation. The relevance of present animal data for human reproduction and infertility is difficult to evaluate. Residues of chlorinated hydrocarbons, for example in serum and follicular fluid of non-exposed women, are reported to be lower (Baukloh *et al.*, 1985; Schlebusch *et al.*, 1989; Van der Ven *et al.*, 1992) than the concentrations measured in the present study. However, residues in the human show a very pronounced variation between individuals. In addition, residues in exposed women, for example after accidental exposure to large amounts of organochlorines, are not known and may reach the concentrations in our animal study. It has to be emphasized that an accumulation *per se* is highly unphysiological and represents a risk, particularly when taking into account that possible long-term effects and relationships with other xenobiotics have not been examined sufficiently yet.

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