Reproductive Effects in F1-generation Rats Perinatally Exposed to PCB126, PCB 118, PCB 153 or 2,3,4,7,8-PnCDF

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1. Introduction

Perinatal exposure to PCBs or TCDD has been found to disturb sexual maturation and reproduction[1,2]. From a cross-fostering study [3] it appears that the effects are mainly induced during exposure *in utero*. In rats effects on the initiation and development of reproductive organs and the sexual differentiation and maturation mainly take place during gestation and early lactation. An explanation for reproductive failure may be the effects of PCBs and related compounds on processes on sex-dependant hepatic cytochrome P450 mediated by the hypothalamic-pituitary axis and the control of growth hormone (GH)[4]. A 2-generation study in rats administered with a range of dose levels of 3,3',4,4',5-PnCB (PCB 126), 2,3',4,4',5-PnCB (PCB 118), 2,2',4,4',5,5'-HxCb (PCB 153) and 2,3,4,7,8-PnCDF on gestation day (GD) 1 was intitiated. In these experiments the development of the F1-generation, sexual maturation, effects on reproductive organs and reproductive capacity in combination with several sex-dependent hepatic cytochrome P450 and steroid hormones in the plasma were studied. Administration of the test substances in a single dose at GD 1 was chosen in order to expose the F0-females well before implantation of the embryos so that the test substances would be distributed evenly over the body of the dam before establishment of the interconnected blood systems of mother and offspring. In this way the offspring would not be exposed to peak values during certain, possibly decisive phases in their development, thus stimulating to some extend the human situation. In the present paper the 2-generation study as such and the fertility, development of the F1-generation and the reproductive capacity are described.

2. Experimental design

Virgin female Wistar rats (SPF-derived, Crl:(WI)WU BR) of about 14 weeks were mated overnight. As soon as a sperm positive vaginal smear was detected the females were housed individually; this day was considered gestation day (GD) 0 and assigned to the experimental groups. On GD 1 a single dose of corn oil (2 ml/kg body weight), control, or of 6, 16, 51, 147, 375 nmol/kg bw (2-121 μg/kg) PCB 126; 2, 7, 22, 75, 251 μmol/kg bw (0.8-81 mg/kg) PCB 118; 53, 147, 200, 402, 768 μmol/kg (19-275 mg/kg) PCB 153 or 1,

4, 12, 43, 131 nmol/kg bw (0.4-0.44 μg/kg) 2,3,4,7,8-PnCDF dissolved in corn oil was administered by gavage. Doses approximately ranged from 0.5 to 45 nmol TEQ/kg bw[5,6]. During the study tap water and a cereal-based rodent diet (meal mash) were provided *ad libitum*. The animals were maintained in a temperature-controlled animal room at 22 ± 2 °C with a relative humidity of 40-80% on a 12 hour-light/dark cycle.
During the study clinical signs were recorded daily. The females were allowed to litter and raise their offspring up to postnatal day (PND) 21, when pups were weaned. At PND 4 the litters were culled to 8 pups , as near as possible to 4 males and 4 females. Body weights and feed consumption were measured on GD 0, 7, 14, 21 and on PND 1, 7, 14 and 21. The offspring was weighed as a litter on PND 1, 4, 7 and 14, and individually on PND 21, and weekly thereafter. The following parameters were recorded for all litters: number of live and dead pups at birth, number of males and females, external abnormalities and mortalities. Preputial separation was examined in male F1-pups at PND 30-33. At weaning (PND 21) and PND 112 six males and six females from different litters per dose group were sacrificed for analyses of cytochrome P450 activities [7,8]. In 16-week old male offspring plasma testosterone levels were measured [8]. The reproductive capacity after *in utero* exposure was tested by mating 12 male and 12 female F1-pups of 2 different dose levels per test substance. In the week prior to mating the vagina of the females was macroscopically examined. The F1-generation males and females (at an age of about 14 weeks) were allowed to mate either with untreated virgin females or males. The resulting F2-generation was killed at PND 4 together with the parental F1-animals. A thorough necropsy was performed. Ovaries, testes, epididymides, prostate and adrenals were weighed. In addition the following organs were fixed either in a neutral, aqueous phosphate buffered 4% solution of formaldehyde (F) or Bouin's fixative (B); ovaries (F), uterus (F), vagina (F), testes (B) epidydimides (B), seminal vesicles (B), prostate (B), coagulating gland (B), penis (F) adrenals (F). Sperm derived from the left cauda epidydimis was analysed for motility, number of spermatozoa and morphological sperm abnormalities. The reproductive organs were examined microscopically.

3. Results

Table 1 Effects in F0-females after a single dose on GD 1

	PCB 118	PCB 126	PCB 153	PnCDF
body weight change GD 1-7	↓	↓↓	↓↓	↓
food consumption GD 1-7	↓↓	↓↓	↓↓	↓↓
females with stillborn pups	-	↑↑	-	-
females with all stillborn pups	-	↑	-	-
litter size	-	↓↓	-	-
pup mortality	-	↑↑	-	-
pup weight	-	↓↓	-	-
pup abnormalities	-	-	-	-

↑ increased, ↑↑ stat.sign. increased, ↓ decreased ,↓↓ stat. sign. decreased

F0-generation
Effects induced in the F0-generation and F1-generation pups are shown in Table 1. After administration on GD 1 of different dose levels of PCB 118, 126, 153 and PnCDF maternal toxicity manifested itself only in a reduction in maternal weight gain and food

consumption during GD 1-7. In the highest dose of the PCB 126 the number of females with stillborn pups/all pups stillborn and the pup mortality during lactation was increased. During lactation pup weights were decreased only in the high and highest PCB126 group. No treatment-related abnormalities were observed in the pups.

Table 2 Effects in F1-males exposed perinatally

	PCB 118	PCB 126	PCB 153	PnCDF
body weight	-	↓↓	-	-
preputial separation	↓	↓	-	-
pre-coital time	-	-	-	-
mating	-	-	-	-
fecundity	-	↓	-	-
litter size	-	↓	-	-
pup mortality	-	↑↑	-	-
organ weights:				
testes	↑↑[a]	↓↓[a]	↓↓[a] ↑[r]	-
prostate	↑↑[ar]	-	-	↑↑[ar]
sperm analysis:				
motility	-	-	-	↓
number of spermatozoa	↓	↓	-	↓
morphology	nd	↑	nd	-
microscopy:				
epididymides[b]	↑	↑	-	↑

↑ increased, ↑↑ stat.sign. increased, ↓ decreased ,↓↓ stat. sign. decreased
[a]: absolute, [r] : relative, nd: not yet determined, [b] : (multi)focal mononuclear-cell infiltrate

F1-generation males

Effects in male F1-generation exposed perinatally are shown in Table 2. No clinical observations were observed in the F1-males during the premating period. Body weights of the perinatally exposed males of the F1-generation of the PCB 126 group were decreased. Observation of the preputial separation in the male F1-pups showed a delay for the PCB 118 and PCB 126 groups. At mating with untreated females no effects were observed in pre-coital time and mating. Fecundity and litter size were only affected in the females mated with the PCB126 exposed males. At necropsy absolute testes weight of the PCB 118 group was increased. A decrease in absolute testes weight was observed in the males of the PCB 126 and PCB 153 group. Absolute and relative prostate weight of the PCB 118 and PnCDF group were decreased. At evaluation of the epididymal sperm an effect was observed in the sperm motility of the PnCDF group, in the number of spermatozoa of the of the PCB 118, PCB 126 and PnCDF group and in the number of spermatozoa with an amorphic head in the PCB 126 group. Sperm morphology of the PCB 118 and PCB 153 are not yet performed.

Microscopical evaluation of the testes, epididymides, seminal vesicles and prostate revealed only a slight increase in the number of animals with epididymides with (multi)focal mononuclear-cell infiltrate in the PCB 118 and PCB 126 groups and an statistically significant increase for this effects in the PnCDF group.

F1-generation females

Effects in female F1-generation exposed perinatally are shown in Table 3. No clinical observations were observed in the F1-females during the premating period. Body weights of the perinatally exposed females of the F1-generation of the PCB 126 group were decreased. About 1 week before the start of the mating period the vagina of all selected perinatally exposed F1-females were carefully examined. Closed vaginas were observed in all exposed groups; this effect was statistically significant in the PCB 126 and PnCDF group. An increase in the number of females with a swollen clitoris was observed in the PCB 126 exposed group. At mating with untreated males pre-coital time was slightly increased in the PCB 118, PCB 126 and PnCDF group. Mating as far as not prohibited by a closed vagina was not affected. The fecundity was decreased in the PCB 118, PCB 126 and PnCDF females. Litter size was reduced in the PCB 126 and PnCDF group. Microscopical evaluation of the ovaries and uterus revealed in the PCB 126 group an slight increase in the number of females with hyperplasia and metaplasia of the uterus-epithelium, and ovaries with a reduced number of corpora lutea.

Table 3 Effects in F1-females exposed perinatally

	PCB 118	PCB 126	PCB 153	PnCDF
body weight	-	↓↓	-	-
vagina: closed	↑	↑↑	↑	↑↑
clitoris: swollen	-	↑↑	-	-
pre-coital time	↑	↑	-	↑
mating	-	-	-	-
fecundity	↓↓	↓↓	-	↓↓
litter size	-	↓↓	-	↓
pup mortality	-	↑↑	-	-
microscopy:				
uterus[c]	-	↑	-	-
ovaries[d]	-	↑	-	-

↑ increased, ↑↑ stat.sign. increased, ↓ decreased ,↓↓ stat. sign. decreased
[c]: focal epithelial hyperplasia/metaplasia, [d]: reduced no. of corpora lutea

4. Discussion and conclusions

Perinatal exposure to PCB153 up to dose levels which induce some maternal toxicity did not affect the reproductive capacity; only a slight decrease in the number of epididymal sperm was observed and some F1-females with a closed vagina were observed.
During lactation of the F0-generation only for PCB 126 effects on the F1-pups such as decreased litter size at birth, pup mortality and decreased pup weight were observed. In the F1-generation the effects of this PCB-congener on parameters concerning male and female reproductive capacity and the development of the reproductive organs were more pronounced when compared to the PCB 118 and the PnCDF groups.
It is unlikely that hepatic P450 expression is involved in the induction of reproduction failures, for in these studies lifelong alterations in hepatic P450 expression due to perinatal interference of PCBs with the imprinting of the P450s were not accompanied by the occurrence of reproductive failures [8]. Moreover, in studies with PCB-congeners that did

induce reproductive effects, P450 induction was not significant. However, of the congeners tested on PND 21 PCB 126 was most potent in induction of CYP1A1 and 1A2 activities[7] and this effect might have played a role in the observed reproductive failures after sexual maturation of the PCB 118-, PCB 126- and PnCDF-exposed animals. Further, no differences in plasma testosterone concentrations were found in exposed F1-males at PND 112[8].

5. References

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