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Comparative Toxicology of Tetrachlorobiphenyls in Mink and Rats

I. Changes in Hepatic Enzyme Activity and Smooth Endoplasmic Reticulum Volume[1]

DEBORAH M. GILLETTE,*[,2] RICHARD D. COREY,†[,3] WILLIAM G. HELFERICH,†[,4]
JAMES M. MCFARLAND,† LINDA J. LOWENSTINE,* DAVID E. MOODY,†
BRUCE D. HAMMOCK,† AND LEE R. SHULL†

*Departments of *Veterinary Pathology and †Environmental Toxicology, University of California, Davis, California 95616*

Comparative Toxicology of Tetrachlorobiphenyls in Mink and Rats. I. Changes in Hepatic Enzyme Activity and Smooth Endoplasmic Reticulum Volume. GILLETTE, D. M., COREY, R. D., HELFERICH, W. G., MCFARLAND, J. M., LOWENSTINE, L. J., MOODY, D. E., HAMMOCK, B. D., AND SHULL, L. R. (1987). *Fundam. Appl. Toxicol.* **8,** 5–14. Mink have been shown previously to be extraordinarily sensitive to polychlorinated biphenyls (PCBs) and related classes of halogenated hydrocarbons. This study explored several aspects of the acute response of mink to two purified tetrachlorobiphenyl (TCB) congeners and compared their response with that of the rat, a less sensitive and more thoroughly studied species. Young female pastel mink and young female Sprague–Dawley rats received three daily intraperitoneal injections with equimolar doses of either 2,4,2′,4′-TCB or 3,4,3′,4′-TCB, and were sacrificed after 7 days. Two control groups were used for each species; one was allowed free access to food and the other was pair-fed to the 3,4,3′,4′-TCB treatment group. Rats remained clinically normal, while mink treated with 3,4,3′,4′-TCB developed severe anorexia, diarrhea, and melena. Both species had significant increases in hepatic cytochrome *P*-450 content and the characteristic shift in the spectral maxima from 450 to 448 nm in the 3,4,3′,4′-TCB- but not in the 2,4,2′,4′-TCB-treated animals. Rats but not mink had increased activities of several hepatic monooxygenases in response to both congeners while microsomal epoxide hydrolase was increased in rats after 2,4,2′,4′-TCB and in mink after 3,4,3′,4′-TCB. Significant increases in the relative volume of smooth endoplasmic reticulum within hepatocytes of 2,4,2′,4′-TCB-treated rats but not mink were confirmed by ultrastructural morphometry. Accumulation of both congeners was greater in adipose tissue than in the liver of either species. In both species, concentrations in adipose tissue were much greater for 2,4,2′,4′-TCB than for 3,4,3′,4′-TCB. PCB toxicosis in mink, as in other species, appeared to be dependent on isomeric arrangement of chlorine substituents. However, unlike other species, the toxicosis was not associated with biochemical or morphological evidence of hepatic enzyme induction. Moreover, the target organ of 3,4,3′,4′-TCB toxicosis in mink was the small intestinal mucosa.

There are 209 theoretically possible congeners of chlorinated biphenyls, which can be grouped according to their ability to act as either cytochrome *P*-450 (phenobarbital type), cytochrome *P*-448 (3-methylcholanthrene type), or mixed-type inducers (Goldstein, 1979; Goldstein *et al.*, 1977; Yoshimura *et al.*, 1979). Numerous structure–activity studies in rodents have demonstrated that congeners having a planar molecular conformation and a strong binding affinity for a cytosolic receptor protein known as the aryl hydrocarbon hydroxylase (Ah) receptor are the most toxic. In addition to their greater toxicity, these congeners such as 3,4,3′,4′-tetrachlorobiphenyl (3,4,3′,4′-TCB) induce cytochrome *P*-448 and a battery of associated hepatic mi-

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[2] Present address: Department of Pathology, New Bolton Center, Kennett Square, PA 19348.

[3] Present address: Stauffer Chemical Co., Richmond, CA 94804.

[4] Present address: Travenol Laboratories, Inc., Round Lake, IL 60073.

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crosomal monooxygenases including aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin *O*-deethylase (ECOD). Toxic effects associated with this group of chlorobiphenyls vary with species but generally include lymphoid atrophy, hepatic necrosis, and chloracne (Parkinson and Safe, 1981; Poland and Glover, 1977). By comparison, nonplanar congeners like 2,4,2',4'-TCB have lower binding affinity for the Ah receptor, are substantially less toxic, and induce cytochrome *P*-450 and associated monooxygenases such as aminopyrine *N*-demethylase (APND) and aldrin epoxidase (AE) (Goldstein, 1979; Goldstein *et al.*, 1977).

Mink (*Mustela vison*) have shown exceptional sensitivity to commercial mixtures of polychlorinated biphenyls (PCBs). This phenomenon was first recognized when Great Lakes fish containing low-level PCB contamination were added to the diet of ranch-raised mink resulting in severe reproductive dysfunction (Bleavins *et al.*, 1980; Ringer and Aulerich, 1981). Experimental oral administration of a technical mixture of PCBs (Aroclor 1254) to mink was reported to have marked toxic effects including gastrointestinal hemorrhage, hepatic fatty change, hepatic necrosis, cerebral edema, disseminated intravascular coagulation, and fibrosis of coronary arteries (Platonow and Karstad, 1973). However, despite their greater sensitivity compared with other mammals mink do not respond to Aroclor administration with the expected increases in cytochrome *P*-448 and associated hepatic monooxygenases including AHH and ECOD (Shull *et al.*, 1982). Moreover, mink were relatively nonresponsive to 3-methylcholanthrene (3MC) (Shull *et al.*, 1983). These findings have raised questions about the oft-demonstrated correlation between induction and toxicity, and about whether mink are also sensitive to the same components of PCB mixtures as other species. Also, the biochemical basis for the unusual sensitivity of mink to chlorinated biphenyls has not been delineated. The present study examined both the toxicologic and hepatic enzymatic responses of mink to two purified PCB congeners, and compared them directly with responses in the rat.

METHODS

Chemicals. The 2,4,2',4'-TCB and 3,4,3',4'-TCB were purchased as purified isomers (greater than 99% pure).[5] For dosing, 2,4,2',4'-TCB was dissolved in sterilized peanut oil to yield a 25-mg/ml solution. Because 3,4,3',4'-TCB was poorly soluble in oil at body temperature, a 25-mg/ml suspension in sterile peanut oil was prepared for dosing.

Experimental design. A total of 24 mink and 24 rats were assigned randomly to one of the four treatment groups: 2,4,2',4'-TCB, 3,4,3',4'-TCB, pair-fed control, or free-choice control. Six animals of each species were in each treatment group. All animals were weighed and 3,4,3',4'-TCB and 2,4,2',4'-TCB groups were injected intraperitoneally with 50 mg/kg of either compound in peanut oil for 3 consecutive days. Both control groups were injected with equivalent amounts of peanut oil alone. Pair-fed controls received only as much food as the 3,4,3',4'-TCB group consumed. Feed intake of each animal was recorded daily. Seven days after the initial injection, all animals were anesthetized in a CO_2 chamber and killed by cervical dislocation. Total body weights and the weights of the brain, spleen, liver, and kidneys were recorded. Both fresh liver and liver snap-frozen in liquid N_2 were collected for hepatic microsomal and cytosolic enzyme assays.

Animals. The mink were 4- to 7-month-old pastel ranch-raised females housed individually in wire cages in climate-controlled rooms, and were fed a complete pelleted mink chow.[6] They were acclimated to this environment for 3 months prior to the onset of the experiment, during which time they were blood tested for Aleution disease and vaccinated for distemper, mink viral enteritis, and *Clostridium botulinum*.[7]

Rats were 45-day-old Sprague–Dawley females[8] individually caged in wire-bottom cages and fed laboratory chow.[9] Rats were housed in a controlled environment with a temperature of 22°C, 50% humidity, and a 12/12-hr light–dark cycle.

Cytochrome determinations and enzyme activities. Microsomes were prepared from either fresh or frozen liver samples by differential centrifugation as described previously (Shull *et al.*, 1982). Microsomal protein concentration was determined by the Biuret method (Gornall *et al.*, 1949).

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[5] Ultrascientific Co., Hope, RI.

[6] National Fur Foods, New Holstein, WI.

[7] Biocom-D, Invernex Veterinary Laboratories, Middleton, WI.

[8] Simonsen Co., Gilroy, CA.

[9] Purina, St. Louis, MO.

Concentrations of cytochrome *P*-450 (448) and b_5 and absorbance maxima of cytochrome *P*-450 were determined by the method of Omura and Sato (1964) using a Cary 219 dual-beam spectrophotometer. Microsomes prepared from fresh liver were analyzed by a standard colorimetric assay for aminopyrine *N*-demethylase (Anders and Mannering, 1966) and a fluorimetric assay for ethoxycoumarin *O*-deethylase (Ullrich and Weber, 1972). Substrate concentrations were 40 mM for aminopyrine and 0.83 mM for ethoxycoumarin. Microsomes prepared from liver stored under liquid N_2 were used to measure aryl hydrocarbon hydroxylase, aldrin epoxidase (AE), and NADPH cytochrome *c* reductase activities. All enzyme assays were validated for time and protein linearity for both animal species. Aryl hydrocarbon hydroxylase activity was measured by a radiometric method (VanCantfort *et al.*, 1977) using 0.158 mM [^{3}H]-benzo(a)pyrene. Aldrin epoxidase was measured by gas chromatographic analysis of the end product, dieldrin, using 0.55 mM aldrin concentration (Kreiger *et al.*, 1976). All monooxygenase enzymes were assayed at a microsomal protein concentration of 0.4 mg/ml except for APND in mink which was 0.8 mg/ml. Incubation time for all monooxygenase assays was 15 min. NADPH cytochrome *c* reductase activity was measured spectrophotometrically (Pederson *et al.*, 1973) using concentrations of 0.1 mg/ml protein and 0.257 mM cytochrome *c*.

Microsomal epoxide hydrolase (mEH), cytosolic epoxide hydrolase (cEH), and glutathione *S*-transferase (GST) were assayed by single-step radiometric partition assays (Gill *et al.*, 1983). In brief, protein was incubated with tritiated substrate (cEH, *trans*-stilbene oxide, pH 7.4; mEH, *cis*-stilbene oxide, pH 9.0; and GST, *cis*-stilbene oxide plus glutathione, pH 7.4) at 37°C for 10–30 min and the epoxide, or epoxide and diol, preferentially extracted with isooctane and hexanol, respectively. For cEH, diluted cytosols were routinely preincubated with 0.5 mM diethylmaleate for 10 min at room temperature to deplete endogenous glutathione (Moody *et al.*, 1986). Cytosolic protein concentration for these assays was quantified using the method of Bradford (1976) as modified by Moody *et al.* (1985).

Morphology. Liver for examination by electron microscopy was fixed in 2.5% gluteraldehyde with 0.1 mM cacodylate buffer at a pH of 7.2–7.4, and postfixed in 1% osmium tetroxide and 2% aqueous uranyl acetate. Tissues were embedded in epon aryldite plastic and thin-sectioned at 90 nm. Sections were mounted on formvar-coated grids, stabilized with approximately 10 Å carbon, and examined on a Zeiss 10A electron microscope.

Hepatocytes immediately adjacent to a central vein were photographed for morphometric determination of smooth endoplasmic reticulum (SER) content. Five fields from each animal were photographed at a magnification of ×1020 and printed at a final magnification of ×2677. Relative volume density of hepatocyte cytoplasm was calculated from these photographs by point counting using a clear overlay with a 7-mm square grid pattern. Ten photographs of hepatocyte cytoplasm from each animal were taken at a magnification of ×10,000 and printed at a final magnification of ×26,775. The same overlay grid was used to determine the relative volume density of SER per unit cytoplasm. Together, values from high- and low-magnification photographs were used to establish the relative volume of SER per cell.

Tissues for light microscopy were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were cut at 6 μm and stained with hematoxylin and eosin.

Quantification of TCBs in tissues. The extraction procedure was adapted from Mills (1959, 1961) and Mull *et al.* (1978). Samples of liver and body fat from both species were thawed at room temperature and minced with scissors. Subsamples of 0.5–0.6 g were combined with 10 ml of hexane and homogenized,[10] the fat until dissolution and the liver for about 1 min. Homogenates were centrifuged[11] at 2000 rpm for 10 min. The hexane supernatant was combined with tube rinsings (three times, 1–2 ml each) in a separate tube and placed in a water bath (35°C) under N_2,[12] and the volume was reduced to about 2 ml (fat) or 3 ml (liver).

Hexane extracts of fat were then transferred to 125-ml separatory funnels using three 1.0-ml hexane rinses producing a total volume of 5.0 ml. Acetonitrile (10 ml) was added and the mixture shaken vigorously; this extraction was repeated five more times. Combined extracts were then evaporated to dryness[13] three times, each time redissolving the residue in 2–3 ml of hexane. Hexane extracts of fat in 2.0 ml were then cleaned up using a silica Sep-Pak.[14] To the dry Sep-Pak was added 10.0 ml of hexane followed by the sample in 2.0 ml of hexane. Sep-Paks were then eluted with three 2.0-ml volumes of hexane. Combined eluate was then analyzed for TCBs by gas chromatography (CG). Mean percentage recovery of spiked TCBs from body fat of control animals was 92.5% (±2.1 SD, $n = 5$) for 2,4,2′,4′-TCB and 92.4% (±2.7 SD, $n = 4$) for 3,4,3′,4′-TCB.

The 3 ml of liver hexane extracts were diluted to 10.0 ml of hexane and then cleaned up with a silica Sep-Pak. To the dry Sep-Pak was added 10.0 ml of hexane. Sep-Paks were eluted with three 1.0-ml volumes of hexane. Combined eluate was then analyzed for TCBs by GC. Mean percentage recovery of spiked TCBs from liver of control animals was 87.5% (±2.5 SD, $n = 5$) for 2,4,2′,4′-TCB and 84.6% (±2.2% SD, $n = 5$) for 3,4,3′,4′-TCB.

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[10] Polytron, Brinkman Instruments, Westbury, NY.

[11] Centra-7, International Equipment Co., Needham Heights, MA.

[12] Meyer N-Evap, Organomation Associates, Inc., Northborough, MA.

[13] Vacuum rotary Calab Model C evaporator, California Laboratory Equipment Co., Oakland, CA.

[14] Waters Associates, Millford, MA.

Gas chromatographic analysis was done with a tritium foil electron capture detector.[15] The glass column was 1.524 m in length and 1.8 mm i.d. The column packing was 1.5% SP 2250/1.95% SP 2401 on 100/120 mesh Supelcoport. Operating temperatures were column temperature, 175°C; detector temperature, 200°C; and injector temperature, 212°C. The carrier gas was nitrogen (99.997% purity) flowing at a rate of 30 ml/min.

Statistical evaluation. Data from cytochrome determinations, enzyme assays, and ultrastructural morphometry were analyzed by a one-way analysis of variance using a Sheffe test for comparisons of means. The level of significance was set at $p = 0.05$.

RESULTS

Feed consumption, body weight, and clinical signs. All rats gained weight during the course of the experiment (Table 1). There were no differences in either food consumption or body weight gain among treatment groups. All rats remained clinically normal during the 7-day experimental period. Likewise, there was no difference in food intake between 2,4,2',4'-TCB-treated mink and free-choice controls (Table 1). However, treatment with 3,4,3',4'-TCB resulted in an immediate and severe depression in feed intake in mink; this effect generally became apparent within 48 hr of the initial injection. Although all mink lost body weight, only the 3,4,3',4'-TCB group and its pair-fed controls lost a significant amount compared with free-choice controls. We attribute the small loss of body weight in 2,4,2',4'-TCB and free-choice animals to stress. All 3,4,3',4'-TCB-treated mink became depressed by the second or third day and had diarrhea, melena, and rough hair coats. Occasionally, particularly toward the end of the experiment, these mink had slight tremors, or rested with head or limbs in a contorted posture. Five of the six 3,4,3',4'-TCB-treated mink were severely depressed by Day 7 and one animal died on Day 6. The results of a battery of hematologic measurements are given in the second paper in this series (Gillette *et al.*, 1987). By contrast, mink given 2,4,2',4'-TCB were normal throughout the course of the experiment.

[15] Varian 1200, Varian Instrument Group, Palo Alto, CA.

TABLE 1

MEAN BODY WEIGHT CHANGES OVER 7 DAYS AND MEAN DAILY FEED CONSUMPTION[1]

	Body weight change (% change over 7 days)	Average feed consumption (g/day)
Mink		
2,4,2',4'-TCB	$-5.96 \pm 2.33^{a,2}$	50.40 ± 8.35^{a}
3,4,3',4'-TCB	-27.72 ± 3.15^{b}	9.52 ± 3.77^{b}
Pair fed	-19.53 ± 0.88^{b}	10.70 ± 3.35^{b}
Free choice	-2.79 ± 1.53^{a}	69.81 ± 10.74^{a}
Rats		
2,4,2',4'-TCB	$+11.59 \pm 3.95^{a}$	20.2 ± 0.56^{a}
3,4,3',4'-TCB	$+7.55 \pm 2.90^{a}$	18.75 ± 1.20^{a}
Pair fed	$+5.50 \pm 2.61^{a}$	17.50 ± 1.17^{a}
Free choice	$+7.96 \pm 1.98^{a}$	19.76 ± 0.59^{a}

[1] $N = 6$ for all treatment groups.

[2] Means (± SE) within a given column and species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

Organ weights. Table 2 shows organ weights which are expressed as a percentage of initial body weight. Average liver weight of 3,4,3',4'-TCB rats was increased significantly compared with that of controls. Average spleen weight of both 2,4,2',4'-TCB- and 3,4,3',4'-TCB-treated rats was increased compared with that of pooled controls. No significant differences occurred in brain or kidney weight in rats or in brain, spleen, kidney, and liver weights of mink.

Hepatic cytochrome concentrations and metabolic enzyme activities. There was a significant increase in hepatic microsomal cytochrome *P*-450 (448) content in both mink and rats that were treated with 3,4,3',4'-TCB (Table 3). By contrast, 2,4,2',4'-TCB caused no such increase in either species. In addition, 3,4,3',4'-TCB caused a significant shift of the absorbance spectral maximum from 450 toward 448 nm in both mink and rats. Cytochrome b_5 content was not affected significantly by either congener in either species. Similarly, no significant changes in NADPH cytochrome *c* reductase activity occurred with either isomer in either species.

In 3,4,3',4'-TCB-treated rats, there was a significant increase in microsomal ECOD

and AHH activities (Table 4). No significant changes in these enzymes occurred with either isomer in mink. APND and AE activities were increased significantly in 2,4,2',4'-TCB-treated rats but not in 2,4,2',4'-TCB-treated mink (Table 4). Microsomal EH activity was increased significantly by 3,4,3',4'-TCB in mink whereas the only significant response in rats was to 2,4,2',4'-TCB (Table 4).

The two TCBs had no significant effect on the two cytosolic enzymes, cEH and GST, in the livers of rats and mink (Table 5). The activity of cEH was decreased slightly in the pair-fed mink, but was not different compared with that in the free-choice control.

Pathologic changes and hepatic SER morphometry. No significant gross or light microscopic abnormalities were seen in the rats. In mink, significant differences between control and treated mink were limited to the small intestine of 3,4,3',4'-TCB-treated animals. These changes, which are described in detail in the second paper of this series (Gillette *et al.*, 1987), consisted of severe necrosis of the epithelium of the small intestine with villous atrophy and fusion.

Significant increases in SER content of hepatocytes occurred only in 2,4,2',4'-TCB-treated rats (Table 6). No significant changes due to TCB treatment in volume density of SER were seen in mink. Table 6 also shows that the mink possessed less than 7% of the hepatocytic SER compared with the rats.

TCB residues in body fat and liver. Table 7 shows the concentrations of both TCB isomers in body fat and liver of both mink and rats. Whereas the concentrations of 3,4,3',4'-TCB in fat were comparable between the species, the concentration of 2,4,2',4'-TCB in rat was about twice that in mink. In rats, the concentration of 3,4,3',4'-TCB was comparable between body fat and liver whereas in mink the liver concentration was 11.5% of the fat concentration. In both rats and mink, more 2,4,2',4'-TCB accumulated in the body fat compared with that in the liver, but the difference between organs was more pronounced in the rat.

DISCUSSION

There were marked differences in the response of the two species to treatment with purified chlorobiphenyls, in terms of both toxicity and hepatic enzyme induction. In rats, it is well established that chlorobiphenyl congeners such as 3,4,3',4'-TCB that induce cytochrome *P*-448 (3MC responsive) also possess greater toxicity than congeners that induce the same cytochrome *P*-450 that responds to phenobarbital (Pb) administration

TABLE 2

MEAN ORGAN WEIGHTS AS A PERCENTAGE OF INITIAL BODY WEIGHT IN TCB-TREATED MINK AND RATS[1]

	Liver	Spleen	Kidney
Mink			
2,4,2',4'-TCB	$3.28 \pm 0.15^{a,2}$	0.37 ± 0.04^{a}	0.31 ± 0.01^{a}
3,4,3',4'-TCB	3.26 ± 0.20^{a}	0.22 ± 0.02^{a}	0.34 ± 0.01^{a}
Pair fed	2.97 ± 0.26^{a}	0.22 ± 0.01^{a}	0.26 ± 0.01^{a}
Free choice	3.15 ± 0.23^{a}	0.38 ± 0.04^{a}	0.29 ± 0.00^{a}
Rats			
2,4,2',4'-TCB	$6.34 \pm 0.04^{a,b}$	0.52 ± 0.03^{a}	0.62 ± 0.04^{a}
3,4,3',4'-TCB	7.20 ± 0.04^{b}	0.50 ± 0.04^{a}	0.62 ± 0.03^{a}
Pair fed	5.19 ± 0.26^{a}	0.26 ± 0.02^{b}	0.60 ± 0.03^{a}
Free choice	5.88 ± 0.54^{a}	$0.35 \pm 0.02^{a,b}$	0.59 ± 0.03^{a}

[1] $N = 6$ for all groups.

[2] Means (±SE) within a given column and species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

TABLE 3

MEAN CONCENTRATIONS OF CYTOCHROMES *P*-450 AND b_5, AND ACTIVITY OF NADPH CYTOCHROME *c* REDUCTASE

	Cytochrome *P*-450[1] (nmol/mg protein)	Absorbance maximum[1] (nm)	Cytochrome b_5[2] (nmol/mg protein)	NADPH cytochrome *c* reductase[3] (nmol/mg protein/min)
Mink				
2,4,2',4'-TCB	$0.13 \pm 0.03^{a,4}$	449.6 ± 0.20^{a}	0.11 ± 0.00^{a}	82.0 ± 7.4^{a}
3,4,3',4'-CB	0.63 ± 0.07^{b}	447.0 ± 0.25^{b}	0.19 ± 0.04^{a}	71.1 ± 5.1^{a}
Pair fed	0.12 ± 0.03^{a}	450.0 ± 0.31^{a}	0.19 ± 0.06^{a}	82.0 ± 7.4^{a}
Free choice	0.18 ± 0.04^{a}	449.5 ± 0.50^{a}	0.12 ± 0.01^{a}	75.4 ± 7.0^{a}
Rats				
2,4,2',4'-TCB	0.44 ± 0.01^{a}	449.2 ± 0.30^{a}	0.38 ± 0.03^{a}	159.2 ± 3.9^{a}
3,4,3',4'-TCB	0.70 ± 0.04^{b}	447.8 ± 0.16^{b}	0.32 ± 0.02^{a}	148.7 ± 15.7^{a}
Pair fed	0.38 ± 0.04^{a}	449.3 ± 0.41^{a}	0.32 ± 0.01^{a}	142.4 ± 8.5^{a}
Free choice	0.29 ± 0.04^{a}	449.3 ± 0.33^{a}	0.24 ± 0.02^{a}	127.7 ± 5.5^{a}

[1] $N = 5$ for pair-fed mink, $N = 4$ for free-choice mink, and $N = 6$ for all other treatment groups.
[2] $N = 6$ for all treatment groups except 3,4,3',4'-TCB-treated mink where $N = 5$.
[3] $N = 6$ for 3,4,3',4'-TCB, 2,4,2',4'-TCB, and pair-fed mink. $N = 5$ for all other treatment groups.
[4] Means (±SE) within a given column and species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

(Goldstein, 1979; Goldstein *et al.*, 1977). Accordingly, 2,4,2',4'-TCB proved relatively innocuous in the mink, whereas 3,4,3',4'-TCB was associated with significant toxicity. The adverse response of mink to technical mixtures of PCBs appears due, as in other species, to the presence of planar 3MC-type congeners like 3,4,3',4-TCB that theoretically are

TABLE 4

ACTIVITIES OF METABOLIC ENZYMES IN HEPATIC MICROSOMES OF TCB-TREATED MINK AND RATS

	Aminopyrine *N*-demethylase[1]	Aldrin epoxidase[2]	Acryl hydrocarbon hydroxylase[2]	Ethoxycoumarin *O*-deethylase[1]	Epoxide hydrolase[3]
	(nmol/mg protein/min)				
Mink					
2,4,2',4'-TCB	$7.36 \pm 0.43^{a,2}$	0.35 ± 0.07^{a}	1.50 ± 0.21^{a}	1.30 ± 0.09^{a}	10.50 ± 0.28^{a}
3,4,3',4'-TCB	2.51 ± 0.27^{b}	0.042 ± 0.01^{b}	1.76 ± 0.21^{a}	0.76 ± 0.21^{a}	23.80 ± 3.60^{b}
Pair fed	$5.46 \pm 1.22^{a,b}$	$0.22 \pm 0.05^{a,b}$	0.95 ± 0.26^{a}	0.68 ± 0.20^{a}	8.75 ± 0.48^{a}
Free choice	$5.45 \pm 0.93^{a,b}$	$0.18 \pm 0.06^{a,b}$	1.02 ± 0.28^{a}	0.79 ± 0.23^{a}	9.53 ± 0.54^{a}
Rats					
2,4,2',4'-TCB	10.44 ± 0.8^{a}	1.99 ± 0.44^{a}	0.85 ± 0.05^{a}	1.1 ± 0.11^{a}	19.1 ± 2.63^{a}
3,4,3',4'-TCB	6.69 ± 0.44^{b}	0.45 ± 0.11^{b}	5.18 ± 0.16^{b}	6.7 ± 0.16^{b}	12.37 ± 1.51^{b}
Pair fed	7.03 ± 0.82^{b}	0.48 ± 0.26^{b}	0.63 ± 0.09^{a}	0.38 ± 0.04^{a}	8.84 ± 0.83^{b}
Free choice	6.31 ± 0.68^{b}	$0.83 \pm 0.31^{a,b}$	0.67 ± 0.09^{a}	0.22 ± 0.05^{a}	6.63 ± 1.21^{b}

[1] $N = 6$ for all except 3,4,3',4'-TCB mink where $N = 5$.
[2] $N = 6$ for 2,4,2',4'-TCB and pair-fed mink. $N = 5$ for 3,4,3',4'-TCB mink and all rats.
[3] $N = 5$ for all animals.
[4] Means (±SE) within a given column and species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

TABLE 5

ACTIVITIES OF HEPATIC CYSTOSOLIC ENZYMES IN TCB-TREATED MINK AND RATS

	n	Protein concentration (mg/ml)	Epoxide hydrolase (nmol/min/mg protein)	Glutathione *S*-transferase (nmol/min/mg protein)
Mink				
2,4,2′,4′-TCB	6	$35.6 \pm 2.2^{a,1}$	0.129 ± 0.011^{a}	8.1 ± 1.3^{a}
3,4,3′,4′-TCB	5	23.1 ± 2.6^{b}	$0.085 \pm 0.011^{a,b}$	7.0 ± 2.8^{a}
Pair fed	6	28.2 ± 4.3^{a}	0.065 ± 0.005^{b}	4.0 ± 0.4^{a}
Free choice	5	31.5 ± 2.9^{a}	$0.099 \pm 0.016^{a,b}$	4.8 ± 0.6^{a}
Rats				
2,4,2′,4′-TCB	5	24.0 ± 2.1^{a}	0.069 ± 0.009^{a}	9.7 ± 1.4^{a}
3,4,3′,4′-TCB	5	26.8 ± 1.5^{a}	0.070 ± 0.017^{a}	10.3 ± 1.1^{a}
Pair fed	5	19.8 ± 1.1^{a}	0.079 ± 0.017^{a}	6.5 ± 0.8^{a}
Free choice	5	25.0 ± 1.2^{a}	0.064 ± 0.019^{a}	7.1 ± 1.1^{a}

[1] Means (±SE) within a given column and species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

capable of binding to the Ah receptor (Poland and Glover, 1977). Under the conditions of our study, neither congener resulted in acute toxic effects in the rat. Other investigators have reported lymphoid necrosis and decreased splenic size in rats administered 3,4,3′,4′-TCB (Yoshimura *et al.*, 1979).

Consistent with previous studies is the increased liver weight seen in the 3,4,3′,4′-TCB- but not 2,4,2′,4′-TCB-treated rats (Goldstein *et al.*, 1977). This was accompanied by a doubling of the hepatic microsomal cytochrome *P*-450 (448) content of the 3,4,3′,4′-TCB rats. As has been shown with 3MC-type induction, there was a significant shift of the reduced CO spectral maximum from an average of 449 to 447 nm. Although increases in cytochrome *P*-450 content in rats given 2,4,2′,4′-TCB have been reported (Goldstein *et al.*, 1977), the dose and time course of our study apparently were not sufficient to elicit this increase.

The induction of specific hepatic monooxygenases that are associated with different cytochromes *P*-450 has been demonstrated several times in the rat by other investigators using purified chlorobiphenyl congeners (Goldstein, 1979; Goldstein *et al.*, 1977; Yoshimura *et al.*, 1979). Similarly, treatment of rats with 3,4,3′,4′-TCB in the present study dramatically increased the activities of monooxygenases (AHH and ECOD) associated with cytochrome *P*-448. By contrast, 2,4,2′,4′-TCB, which lacks planarity and hence the ability to bind the Ah receptor, induced monooxygenases (APND and AE) typically associated with cytochrome *P*-450.

As in the rat, 3,4,3′,4′-TCB in the mink increased the concentration of hepatic microsomal cytochromes *P*-450 and shifted the reduced CO spectral maximum to 447 nm. Aside from this response, the mink differed markedly from the rat. The 3,4,3′,4′-TCB isomer caused no significant increase in liver weight nor in the activities of AHH or ECOD although resting levels of these monooxygenases were higher in the mink than in the rat. The 2,4,2′,4′-TCB isomer was equally ineffective as an inducer of APND and AE in the mink. Phenobarbital-type inducers are also known to increase the content of SER in hepatocytes (Staubli *et al.*, 1969), with the greatest induction occurring in centrilobular hepatocytes (Massey and Butler, 1979). Morphometric determinations confirmed that 2,4,2′,4′-TCB treatment in the rats caused a dramatic increase in the relative volume of the SER. As would be expected of a 3MC-type inducer (Hodgson, 1980) 3,4,3′,4′-TCB did not stimulate SER proliferation in rats.

TABLE 6

MEAN VOLUME DENSITY OF HEPATOCYTIC SMOOTH ENDOPLASMIC RETICULUM IN TCB-TREATED MINK AND RATS[1]

	Relative percentage per cell
Mink	
2,4,2′,4′-TCB	$1.57 \pm 0.12^{a,2}$
3,4,3′,4′-TCB	1.03 ± 0.08^{a}
Pair fed	1.07 ± 0.34^{a}
Free choice	0.92 ± 0.03^{a}
Rats	
2,4,2′,4′-TCB	34.13 ± 1.31^{a}
3,4,3′,4′-TCB	17.90 ± 1.61^{b}
Pair fed	13.37 ± 0.71^{b}
Free choice	14.46 ± 1.68^{b}

[1] $N = 5$ for all treatment groups.
[2] Means (±SE) within species having the same superscript (*a* or *b*) are not significantly different ($p \leq 0.05$).

We were unable to demonstrate by morphometry any significant increase in relative SER content in mink hepatocytes after treatment with either congener.

These results agree with observations made previously on the dramatic toxicity of technical PCB mixtures in mink and the limited ability of this species to respond to inducers including PCBs. Shull *et al.* (1982) administered a technical PCB mixture (Aroclor 1254) to mink and were not able to demonstrate a significant increase in AHH, ECOD, ethylmorphine *N*-demethylase, or hexobarbital hydroxylase (HH) over pair-fed controls, nor did they observe any changes in cytochrome b_5 concentrations. Data collected by the same investigators from mink treated with either Pb or 3MC suggested that the mink has only a limited ability to respond to hepatic monooxygenase inducers (Shull *et al.*, 1983). They reported that 3MC caused a significant increase in cytochrome *P*-448 and b_5 and moderate increases in AHH and ethoxyresorufrin *O*-deethylase (EROD); Pb significantly increased APND but not HH. Failure of 2,4,2′,4′-TCB to elicit increases in SER profiles in hepatocytes is consistent with previous observations (Shull *et al.*, 1983) that Pb was ineffective in promoting SER proliferation.

Aulerich *et al.* (1985) compared the response of adult female mink with purified hexachlorobiphenyls (HCBs) administered orally. Consistent with our findings, the 3MC-type congener 3,4,5,3′,4′,5′-HCB was considerably more toxic than the Pb-type congeners 2,4,5,2′,4′,5′ or 2,3,6,2′,3′,6′-HCB. None of the hexachlorobiphenyls caused an increase in liver weight, but induction of certain hepatic monooxygenases did occur. Aryl hydrocarbon hydroxylase but not EROD was increased significantly by 3,4,5,3′,4′,5′-HCB. Aminopyrine *N*-demethylase was induced by 2,4,5,2′,4′,5′ but not by 2,3,6,2′,3′,6′-HCB. The ability of the hexachloro- but not the tetrachlorobiphenyls to induce some hepatic monooxygenases in the mink may relate to the presence of the additional chlorines on the ring, perhaps resulting in greater retention of these more highly chlorinated congeners, or may be influenced by the longer duration of exposure (2–3 months) to the hexachloro congeners.

Residues in fat and liver varied with the congener as well as with the species. Adipose and hepatic tissue concentrations of 3,4,3′,4′-TCB in female Sprague–Dawley rats apparently are dependent on both the absolute dose and the dosing schedule of the com-

TABLE 7

CONCENTRATIONS OF 2,4,2′,4′-TCB AND 3,4,3′,4′-TCB IN POSTMORTEM FAT AND LIVER[1]

	Fat (ppm)	Liver (ppm)
Mink		
2,4,2′,4′-TCB	389 ± 42^{2}	38 ± 9^{4}
3,4,3′,4′-TCB	139 ± 43^{3}	16 ± 7^{5}
Rats		
2,4,2′,4′-TCB	747 ± 104	28 ± 15^{4}
3,4,3′,4′-TCB	148 ± 35	138 ± 31^{3}

[1] Tissue samples were stored frozen until analyzed.
[2] Mean (±SE) of six analyses except where indicated.
[3] $N = 5$.
[4] $N = 4$.
[5] $N = 3$.

pound (Clarke *et al.*, 1983). Shimada and Sawabe (1984) compared the distribution of radiolabeled 2,4,2′,4′-TCB and 3,4,3′,4′-TCB in male Sprague–Dawley rats and reported a much greater accumulation of 2,4,2′,4′-TCB vs 3,4,3′,4′-TCB in adipose tissue than in liver, a finding consistent with our results in both species. Although they reported similar concentrations of both congeners in rat liver, they noted that covalent binding of 3,4,3′,4′-TCB to liver was much greater than binding of 2,4,2′,4′-TCB and suggested that this may explain the greater toxicity of this congener. A study conducted in chick embryos showed that although 3,4,3′,4′-TCB was at least 10,000 times more toxic to the embryos than was 2,4,2′,4′-TCB, no differences in autoradiographic distribution of the congeners could be found to explain differences in toxicity (Brunstrom and Barnerud, 1983). Since our extraction methods permitted measurement of only unbound TCB, the correlation between toxicity of 3,4,3′,4′-TCB and degree of tissue binding remains unknown.

The target organ of 3,4,3′,4′-TCB toxicosis in the mink was the small intestinal mucosa. Intestinal disease of this type has not been reported previously as a feature of PCB intoxication in postnatal animals of any species. The intestinal pathology was quite severe, present diffusely throughout the small intestine, and appeared to be the basis of the extreme sensitivity of mink to different PCB congeners. The pathologic changes in the mink of this study are described in detail in the subsequent presentation in this journal (Gillette *et al.*, 1987).

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