0953

Ref

TERATOLOGY 29:21–27 (1984)

A Teratological Evaluation and Analysis of Fetal Tissue Levels Following Administration of Tetrachlorobenzene Isomers to the Rat

S. KACEW, J.A. RUDDICK, M. PARULEKAR, V.E. VALLI, I. CHU, AND D.C. VILLENEUVE
Department of Pharmacology, Faculty of Health Sciences, School of Medicine, University of Ottawa, (S.K., M.P.); Environmental and Occupational Toxicology, Environmental Health Centre, Tunney's Pasture, Ottawa, Ontario (J.A.R., I.C., D.C.V.); Biopath Analysts Ltd., Guelph, Ontario (V.E.V.)

ABSTRACT Three tetrachlorobenzene (TCB) congeners (1,2,3,4-, 1,2,3,5-, and 1,2,4,5-) were administered daily by gavage to pregnant Sprague–Dawley rats at levels of 50, 100, or 200 mg/kg from day 6 through day 15 of gestation. Mothers were sacrificed on day 21 of gestation and the pups removed by cesarian section for teratological evaluation. Administration of 1,2,3,4- and 1,2,3,5-TCB failed to alter maternal body weight, organ weights, hematological, or the biochemical parameters studied. The highest dose level of 1,2,4,5-tetrachlorobenzene caused maternal death in nine of ten animals. In addition it induced mixed function oxidases and increased serum cholesterol values at 50 and 100 mg/kg. There was a decrease in the number of fetuses at the highest dose levels of 1,2,3,4- and 1,2,3,5-TCB and at the lowest dose level of the 1,2,4,5- congener. None of the congeners produced any anomalies. There were no treatment-related histopathological changes in either the mothers or fetuses. Residues of all three congeners were found in maternal and fetal tissues but generally the amounts of the 1,2,4,5- isomer were about 100 times higher than the other two.

Tetrachlorobenzene (TCB) comprises three isomers (1,2,3,4, 1,2,3,5, and 1,2,4,5). These congeners have been identified as contaminants in fish of the Great Lakes in North America (Konasewich et al., '78 and Veith et al., '79). The 1,2,4,5-TCB congener was found to be present in adipose tissue of residents in Tokyo (Morita et al., '75), but the authors were unable to identify the source and route of TCB emission into the environment. In the past, 1,2,4,5-TCB was used mainly in the synthesis of 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxyacetic acid. It was suggested that general population exposure resulted from manufacturing, industrial use, processing, and disposal (EPA, '80). The presence of these chemicals in the environment and their identification in human tissues suggested the need for further experimentation to determine the effect of TCB.

At present, data are not available on the effects of TCB during pregnancy. Studies were thus undertaken to examine the influence of various dosages of the isomers on pregnant rats and their progeny. In addition to hematological, histological, biochemical and teratological evaluations, the concentration of each of the three TCB isomers was determined in selected fetal and maternal tissues.

MATERIALS AND METHODS

Animal treatment

Female Sprague–Dawley rats weighing approximately 175 g and proven male sires (300 gm) were purchased from Charles River Breeding Laboratories (Portage, MI). All animals had free access to feed (Purina Rodent Laboratory Chow, Toronto, Ontario) and water throughout the experimental period. Animals were used after a 7-day period of acclimatization, after which two females were mated with one male. The following

Received July 14, 1982; accepted July 18, 1983.

1984 ALAN R. LISS, INC.

9361

morning vaginal smears were carried out on each female rat and a positive smear was used to indicate the first day of gestation.

Tetrachlorobenzene congeners (1,2,3,4-, 1,2,3,5-, and 1,2,4,5-TCB, 98–99% pure) purchased from Aldrich Chemical Company (Milwaukee, WI), were recrystallized from 95% ethanol and identified as 99.5% pure by gas chromatography. Each congener was dissolved in corn oil and administered by gavage at dosages of 50, 100, or 200 mg/kg in a constant volume of 0.5 ml/100 gm body weight of the dam. Corresponding controls received an equal volume of the corn oil. The starting number of pregnant rats in each group was ten. Starting on the sixth day of gestation, beginning at 0900 hr, either corn oil or a TCB congener was administered daily through to the 15th day of gestation. On day 21 of gestation, the animals were weighed, anesthetized with ether, exsanguinated via the abdominal aorta, and the pups were removed by cesarean section and weighed individually. The dams were then reweighed and maternal liver, brain, kidney, perirenal fat, spleen, and heart were excised and weighed. Animals that died during the experiment were autopsied and subjected to gross and microscopic pathological examination.

Hematological evaluation

At necropsy, the following hematological parameters were determined for the dams: hemoglobin concentration, hematocrit value, erythrocyte count, total and differential counts of leucocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Biochemical determinations

Serum from each dam was used to give profile to selected parameters for serum chemistry analysis using a SMA 12/60 microanalyzer (Technicon, Montreal, Quebec). Sodium, potassium, inorganic phosphorus, total bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase (GOT), total protein, calcium, cholesterol, glucose, uric acid, and lactic dehydrogenase (LDH) were measured in this fashion.

Weighed portions from the central lobe of the maternal liver were homogenized in 0.15 M KCl, pH 7.4 (1 gm/2.5 ml). Hepatic homogenates were centrifuged at 10,000 g for 15 min at 0°C and the supernatant obtained was used for enzymatic analysis. Aniline hydroxylase activity (AH) was determined by an automated method based on the procedure of Fouts ('63); aminopyrine-N-demethylase (AP) was determined by an automated method based on the procedure of Cochin and Axelrod ('59), and protein concentrations were determined by the biuret method of Gornall et al ('48) as modified by Becking ('73).

Residue analysis

At termination of the study, portions of maternal kidney, brain, spleen, heart, liver, and perirenal fat between 100–400 mg, were weighed for residue analysis. In addition, a fetus from each litter and the entire liver and brain of a litter mate were removed for TCB residue analysis. All tissues were frozen immediately in liquid nitrogen and stored at −20°C until the day of analysis. The TCB congeners were extracted according to a modified method of Hallett et al ('78). Samples of tissues were homogenized in 8 ml of a mixture of acetonitrile:distilled water (1:1). The homogenate obtained was mixed with 4 ml of *n*-hexane, shaken for 30 min, and then centrifuged at 1000 g for 15 min at 22°C. An aliquot of the *n*-hexane layer was removed and washed with an equal amount of concentrated H_2SO_4. The resultant solution was analyzed using a Hewlett Packard 5830A gas chromatograph fitted with a ^{63}Ni electron-capture detector and a glass column (100 × 6.25 mm O.D.) packed with 4% SE-30 and 6% QF-1 on Supelcoport. The gas chromatographic conditions were as follows: oven temperature, 275°C; detector temperature, 300°C; carrier gas, nitrogen; flow rate, 48 ml/min. Recovery ranged from 75–95% depending upon the isomer and the detection limit was 0.05 ppm. Retention times for 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-TCB were 4.80, 3.81, and 3.80, respectively.

Teratological evaluation

At necropsy all fetuses were examined grossly for birth defects. Only live fetuses, however, were counted and evaluated for terata. Approximately two-thirds of the fetuses from each litter were processed for skeleton examination while the remainder were fixed in Bouin's fluid for visceral examination. The visceral anomalies were searched for by dissecting and razor sectioning the fetuses; the skeleton anomalies by stereoscopic examination.

Histopathology

The histological procedure used to examine the maternal heart, brain, pituitary, eye,

thyroid, parathyroid, trachea, bronchi, lung, thymus, stomach, small and large intestine, pancreas, liver, kidney, spleen, adrenal, skeletal muscle, peripheral nerve, skin, bone marrow, ovary, uterus, and bladder has been previously described (Villeneuve et al, '79). In addition, entire fetuses were embedded in paraffin, serial sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical analysis

The data of each parameter in this study were subjected to a simple one-way analysis of variance. When significant differences ($p \leqslant 0.05$) were indicated, the data were subjected to Duncan's Multiple Range Test (SPSS version 8.1) in order to determine the groups that were significantly different.

RESULTS

Mortality and clinical symptoms were limited to pregnant rats that received 200 mg/kg of 1,2,4,5-TCB; 9 of these 10 animals died prior to day 21 of gestation. The mean time of death for these dams occurred about 6.5 days after the start of dosing. At necropsy these animals had signs of severe alimentary toxemia with uterine vascular hemorrhages. Hemorrhages were also present in the lumen of the small intestine and thoracic cavities. The cause of death was attributed to circulatory collapse. In the other groups of animals there were no significant differences in body weight or body weight gain. No marked difference was noted in the absolute weight of maternal brain, heart, kidney, liver, and spleen, or in their relative weight as a percentage of the post-cesarian body weight.

The values for the hematological parameters determined were within the normal ranges established for the pregnant Sprague–Dawley rat in our own laboratories (hemoglobin, 10.6 ± 0.8 gm/100 ml; hematocrit, $31.4 \pm 2.7\%$; erythrocyte count, $5.7 \pm 0.5 \times 10^6/mm^3$; leucocyte count, $5.8 \pm 1.3 \times 10^3/mm^3$; MCH, 18.6 ± 0.9 μg; MCV, 55.3 ± 2.5 μm^3; MCHC, $33.6 \pm 0.5\%$; and platelets, 12.7 ± 2.1).

Statistical analysis did not reveal any significant differences in leucocyte differential counts between treatment and control groups although there was a trend towards greater numbers of eosinophils in all TCB-treated groups. As shown in Table 1, treatment with each of the three TCB congeners at each concentration tended to lower the level of platelets and in the case of 200 mg/kg 1,2,3,4-TCB the decrease in platelet count was significant.

Data in Table 1 also indicate the effects of the various dosages of the three TCB congeners on selected biochemical parameters. Irrespective of the TCB isomer administered, no significant differences were found between control and treated groups in serum alkaline phosphatase, uric acid, LDH, total protein, glucose, sodium, potassium, calcium, inorganic phosphorus, bilirubin, GOT, and hence the results were not included in Table 1. Although no marked change was noted in serum cholesterol for dams given either 1,2,3,4- or 1,2,3,5-TCB, a significant increase occurred in the 50 and 100 mg/kg of 1,2,4,5-TCB groups. A similar increase in AH activity was found in the hepatic supernate from dams administered 50 or 100 mg/kg 1,2,4,5-TCB. While both dosages of 1,2,4,5-TCB tended to elevate liver AP activity, the increase was statistically significant only in the case of the lower dose. In all treatment groups the protein concentration in the liver remained insignificantly different from the control groups.

The maternal TCB tissue residue data are presented in Table 2. TCB isomers were identified in the control tissues as well as in all maternal organs examined. Levels of 1,2,3,4- and 1,2,3,5-TCB were similar for each tissue. The 1,2,4,5-TCB isomer was identified to a far greater extent than the two other congeners. In contrast to 1,2,3,4- or 1,2,3,5-TCB, the presence of 1,2,4,5-TCB was dose-related with the highest levels present in all tissues of the dam given 200 mg/kg compound. Irrespective of the dose of 1,2,4,5-TCB administered the greatest concentration of the isomer occurred in perirenal fat.

Data in Table 3 illustrates the TCB residue levels in the liver, brain, and whole body of the fetus. In general, treatment with 1,2,3,4- or 1,2,3,5-TCB did not result in any significant increase of these isomers in the samples analyzed. As in the case of maternal tissues, fetuses of dams receiving 1,2,4,5-TCB had the highest amounts of TCB. The appearance of 1,2,4,5-TCB, in general, occurred in a dose-dependent manner with the liver having the highest concentration followed by the brain. Amounts in the whole fetus also illustrated dose-dependency.

Results in Table 4 indicate that while the 50 and 100 mg/kg dosages of 1,2,3,4-TCB did not alter the litter size, a significant decrease in the mean number of live fetuses per litter did occur in dams given the 200 mg/kg dose. Similarly, only the 200 mg/kg treatment of 1,2,3,5-TCB was found to reduce the number of live fetuses whereas the lower dosages of

TABLE 1. Effect of tetrachlorobenzene isomers on selected hematological and biochemical parameters[1]

Treatment[2]	Dose (mg/kg)	N	Platelets ($\times 10^6/mm^3$)	Serum cholesterol (mg/100 ml)	Liver AH (μmoles/hr/mg protein)	Liver AP (μmoles/hr/mg protein)
Control	0	8	13.5 ± 1.6 (8)	82 ± 9(7)	11.9 ± 3.8(3)	16.2 ± 2.9(3)
1,2,3,4-TCB	50	8	12.9 ± 1.8 (8)	88 ± 10(8)	13.8 ± 1.9(4)	16.7 ± 2.5(4)
	100	8	12.8 ± 3.0 (8)	78 ± 12(7)	12.5 ± 2.4(6)	16.7 ± 4.6(6)
	200	5	10.0 ± 1.0 (5)[3]	97 ± 18(5)	15.9 ± 1.7(5)	20.5 ± 5.0(5)
1,2,3,5-TCB	50	10	11.9 ± 1.9(10)	90 ± 8(8)	14.0 ± 2.9(9)	20.1 ± 5.1(9)
	100	7	11.5 ± 1.1 (7)	88 ± 12(7)	14.2 ± 2.1(7)	18.6 ± 2.5(7)
	200	6	11.3 ± 1.4 (6)	83 ± 9(6)	15.2 ± 3.0(6)	18.7 ± 2.4(6)
1,2,4,5-TCB	50	8	10.8 ± 1.1 (8)	105 ± 19(7)[3]	19.9 ± 6.4(7)[3]	26.5 ± 7.7(7)[3]
	100	7	11.7 ± 2.1 (7)	103 ± 20(7)[3]	19.9 ± 6.0(7)[3]	24.7 ± 9.3(7)
	200	1	10.8	ND	32.6	47.3

[1]Values represent mean ± SD of the number of animals indicated in parentheses. Pregnant Sprague–Dawley rats were administered PO daily either 50, 100, or 200 mg/kg of 1,2,3,4-TCB, 1,2,3,5-TCB, or 1,2,4,5-TCB in corn oil from days 6 through 15 of gestation and killed on day 21 of gestation. Data for 200 mg/kg 1,2,4,5-TCB is from one rat that survived and is presented as a point of information. ND, not determined; N, number of pregnant rats.
[2]Differences between sample size (in bracket) and group size (N) is due to lost samples.
[3]Statistically significant difference when compared with the values of controls ($p < 0.05$).

TABLE 2. Tetrachlorobenzene residues in tissues of adult female rats[1]

Treatment	Dose (mg/kg)	Concentration of chemical (ppm, μg/g)					
		Kidney	Brain	Perirenal fat	Heart	Liver	Spleen
Control (Corn oil)	0	0.16 ± 0.21 (8)	0.12 ± 0.11 (8)	2.88 ± 2.37 (8)	0.12 ± 0.08 (8)	0.17 ± 0.21 (8)	0.09 ± 0.10 (8)
1,2,3,4-TCB	50	0.51 ± 0.22 (7)	0.44 ± 0.18 (8)	16.0 ± 5.7 (8)	0.40 ± 0.17 (8)	0.56 ± 0.24 (8)	0.33 ± 0.18 (8)
	100	0.59 ± 0.37 (7)	0.41 ± 0.15 (8)	15.2 ± 6.3 (8)	0.40 ± 0.16 (8)	0.54 ± 0.21 (8)	0.34 ± 0.20 (8)
	200	0.59 ± 0.37 (5)	0.28 ± 0.09 (5)	11.3 ± 3.1 (5)	0.28 ± 0.07 (5)	0.35 ± 0.12 (5)	0.16 ± 0.06 (5)
1,2,3,5-TCB	50	0.30 ± 0.13(10)	0.36 ± 0.23(10)	17.0 ± 9.7 (10)	0.28 ± 0.15(10)	0.45 ± 0.25(10)	0.17 ± 0.08(10)
	100	0.33 ± 0.22 (7)	0.36 ± 0.27 (7)	14.2 ± 4.3 (7)	0.28 ± 0.23 (6)	0.42 ± 0.34 (7)	0.25 ± 0.27 (7)
	200	0.51 ± 0.64 (6)	0.71 ± 0.81 (6)	56.3 ± 49.5 (6)	0.51 ± 0.70 (6)	0.78 ± 1.11 (6)	0.45 ± 0.54 (6)
1,2,4,5-TCB	50	39.1 ± 13.6 (8)	32.2 ± 9.8 (8)	1128.4 ± 375.1 (8)	30.0 ± 9.2 (8)	32.8 ± 4.6 (8)	23.6 ± 5.3 (8)
	100	51.1 ± 22.1 (7)	52.0 ± 13.5 (7)	2219.3 ± 368.9 (7)	48.8 ± 9.6 (7)	63.5 ± 14.7 (7)	50.5 ± 41.4 (7)
	200	64.7 (1)	76.0 (1)	3312.4 (1)	71.0 (1)	88.9 (1)	42.1 (1)

[1]Values represent mean ± SD of the number of animals indicated in parentheses. For experimental details, see footnote to Table 1.

TABLE 3. Residual levels of tetrachlorobenzene isomers found in fetal tissues and whole body[1]

Treatment	Dose (mg/kg)	Level of Chemical: Liver (ppm, μg/ml)	Brain (ppm, μg/ml)	Whole body (total μg)
Control (Corn oil)	0	0.14 ± 0.11 (7)	0.09 ± 0.08 (8)	0.17 ± 0.10(7)
1,2,3,4-TCB	50	0.26 ± 0.14 (8)	0.22 ± 0.27 (8)	1.0 ± 0.3 (8)
	100	0.27 ± 0.10 (8)	0.14 ± 0.08 (8)	1.1 ± 0.5 (8)
	200	0.12 ± 0.04 (5)	0.18 ± 0.21 (5)	0.7 ± 0.3 (4)
1,2,3,5-TCB	50	0.14 ± 0.07(10)	0.05 ± 0.02(10)	0.6 ± 0.2(10)
	100	0.13 ± 0.13 (7)	0.07 ± 0.06 (7)	0.71 ± 0.5 (7)
	200	0.13 ± 0.10 (5)	0.16 ± 0.28 (6)	1.5 ± 2.0 (5)
1,2,4,5-TCB	50	23.4 ± 15.3 (8)	16.4 ± 14.90 (7)	70.8 ± 30.1 (7)
	100	36.8 ± 21.3 (6)	17.6 ± 7.3 (7)	108.8 ± 28.9 (7)
	200	42.8 (1)	26.9 (1)	154.0 (1)

[1]Values represent the mean ± SD of the number of animals indicated in parentheses. For experimental detail see footnote to Table 1.

TABLE 4. Effect of various tetrachlorobenzene congeners on the number of live fetuses and body weight[1]

Treatment	Dose	Number of fetuses per dam	Number of resorptions	Average pup weight (gm)	Maternal weight gain (gm)
Control (Corn oil)	0	14.0 ± 4.2 (8)	0.6 ± 0.9 (8)	5.3 ± 0.2 (8)	49 ± 19 (8)
1,2,3,4-TCB	50	12.6 ± 2.6 (8)	1.0 ± 1.1 (8)	5.5 ± 0.2 (8)	56 ± 18 (8)
	100	13.8 ± 1.2 (8)	0.4 ± 0.5 (8)	5.3 ± 0.5 (8)	40 ± 17 (8)
	200	9.6 ± 3.8 (5)[2]	2.4 ± 2.5 (5)	5.3 ± 0.3 (5)	52 ± 28 (5)
1,2,3,5-TCB	50	14.2 ± 2.5(10)	1.2 ± 1.8(10)	5.1 ± 0.4(10)	38 ± 7(10)
	100	14.7 ± 2.0 (7)	0.3 ± 0.8 (7)	5.3 ± 0.2 (7)	42 ± 14 (7)
	200	9.7 ± 4.2 (6)[2]	1.2 ± 1.2 (6)	5.2 ± 0.7 (6)	34 ± 15 (6)
1,2,4,5-TCB	50	8.3 ± 5.5 (8)[2]	2.1 ± 1.5 (8)	5.1 ± 1.0 (6)	41 ± 27 (8)
	100	13.5 ± 2.1 (7)	1.7 ± 1.0 (7)	4.6 ± 1.1 (7)	31 ± 33 (7)
	200	12.0	1.0 (1)	5.2	37

[1]Values represent mean ± SD of the number of mothers shown in parentheses. For experimental detail, see footnote to Table 1.
[2]Statistically significant difference when compared with the values of control ($p < 0.05$).

this isomer were without a marked affect. Although the 50 mg/kg amount of 1,2,4,5-TCB significantly decreased the mean number of fetuses, this effect was not observed at 100 mg/kg or in the one surviving litter at 200 mg/kg. The number of resorptions was not increased by treatment although there was a trend toward a higher number in the highest dose group receiving 1,2,3,4-TCB. Irrespective of the treatment regimen, average pup weight and maternal weight gain were not significantly different from control values.

Evaluation of the pups for anomalies revealed that the chemicals were without any marked effect. Only one pup obtained from the 200 mg/kg group receiving 1,2,3,5-TCB had a birth defect. It was observed at necropsy to have a subcutaneous edema around the head and neck and had a gastrochisis. A talipes was present in the left hind limb that was vividly evident in the skeleton preparation. The ribs on the right side were also extensively fused. The predominant findings in all other pups were a minor delayed osteogenesis in the cranium, sternebrae, small pup size, and presence of a 14th rib (Table 5).

Histopathological changes in the pregnant females were few and not considered to be significant. Fetal histopathological changes

TABLE 5. Teratological data for pups from dams treated with tetrachlorobenzene isomers

	Visceral			Skeleton	
Treatment	Dose (mg/kg)	Pups examined	Observations	Pups examined	Observations
Control	0	27		62	sternum (4/4), 14th rib (1/1)
1,2,3,4-TCB	50	24	small pup (1/1)[1]	53	sternum (4/3)
	100	28	small pup (1/1)	58	sternum (6/3), 14th rib (2/2)
	200	9	small pup (1/1)	25	14th rib (1/1)
1,2,3,5-TCB	50	35		77	small pups (2/2), sternum (8/5), 14th rib (3/3), cranium (2/1)
	100	25	small pup (1/1), hydronephrosis (1/1)	58	small pup (1/1), sternum (1/1), 14th rib (3/2)
	200	10		30	small pup (1/1), sternum (3/2), 14th rib (1/1), wavy rib (1/1), talipes (1/1)
1,2,3,5-TCB	50	10	small pup (3/1)	32	small pup (7/2), sternum (7/3), 14th rib (3/3), cranium (3/3)
	100	25	small pup (4/1)	50	small pup (8/3), sternum (5/1), 14th rib (2/2), wavy ribs (1/1), cranium (1/1)
	200	3	hydronephrosis (1/1)	6	

[1]Number of pups showing effect/number of litters affected. For experimental detail, see footnote to Table 1.

were considered to be incidental in nature and not dose-dependent. These were hematopoietic findings in the liver and bone marrow and consisted of dyserythropoesis characterized by multinucleated red cell precursors in which the nuclei had marked anisokaryosis. These changes occurred predominantly in marrow and in liver and to a lesser extent in the spleen and other tissues where extramedullary hematopoiesis was found. Hepatic changes also consisted of a mild reduction in perinuclear clear cytoplasm, possibly as a result of a decrease in lipid and glycogen.

DISCUSSION

At present little data are available on the effects of TCB isomers on mammalian reproductive function. In this study, treatment of pregnant rats with three different dosages of 1,2,3,4- and 1,2,3,5-TCB produced no dose-related changes in serum or hematological parameters. However, the finding that the number of fetuses were reduced in dams receiving a 200 mg/kg dose of these two congeners indicates that the fetus may be more susceptible than the dam. In a recent study, Braun et al ('78) found that administration of 1,2,4,5-TCB for 2 years to beagle dogs elevated serum alkaline phosphatase and total bilirubin, but was accompanied by no marked change in GOT, glucose, and blood urea nitrogen. In the present study, treatment with 50 or 100 mg/kg of 1,2,4,5-TCB failed to alter serum chemistry parameters markedly except to produce a 25% increase in cholesterol levels. As suggested by Braun et al ('78), the observed increase in serum cholesterol may be an indication of slight impairment of liver function in dogs; our histopathological observations did not reveal any cellular changes. Of the three congeners tested in this study, 1,2,4,5-TCB was the most toxic as evidenced by the death of nine out of ten dams given the 200 mg/kg dose. Similarly, the 1,2,4,5-TCB isomer appeared more fetotoxic in comparison to the other two congeners as reflected by the finding that only 50 mg/kg treatment reduced the mean number of live fetuses while 200 mg/kg of 1,2,3,4- and 1,2,3,5-TCB was needed to induce a similar effect. Although a decrease in the number of fetuses was observed at 50 mg/kg of the 1,2,4,5 congener, a similar effect was not observed at 100 mg/kg. This is difficult to explain since tissue analysis confirmed the levels of 1,2,4,5-TCB were greater in the 100 mg/kg group.

A variety of chemicals are known to induce hepatic microsomal drug-metabolizing enzymes (Conney, '67). Among various chlorinated benzenes examined, Ariyoshi et al ('75a) found that daily, oral administration of 250 mg/kg 1,2,4,5-TCB for 3 days increased the activities of AH and AP in the livers of

female Wistar rats. In a subsequent study, Ariyoshi et al ('75b) found that treatment with 250 mg/kg/day for 3 days with 1,2,3,4- and 1,2,3,5-TCB as well as 1,2,4,5-TCB elevated hepatic AP activity, but not AH activity. In view of the fact that 1,2,4,5-TCB increased hepatic AH activity in one study (Ariyoshi et al, '75a) yet failed to affect enzymic activity subsequently (Ariyoshi et al, '75b) indicates that further investigations are needed to determine the influence of TCB isomers on microsomal enzymes. In our experiment only the 1,2,4,5-TCB congener induced liver AH and AP in pregnant rats. Our data support the view that stimulation of hepatic microsomal drug degradation is related to the chemical structure of TCB and not the total number of chlorine atoms as suggested by Ariyoshi et al ('75a).

Tissue analysis revealed the presence of all three TCB isomers in maternal kidney, brain, perirenal fat, heart, liver, and spleen; however, only 1,2,4,5-TCB was observed to be present in a dose-dependent manner and in greater quantities. The presence of TCB isomers in the control samples may be due to environmental contamination or volatilization of the test compound. Irrespective of the isomer studied, the highest TCB concentration was always present in perirenal fat. As in the case of dams, fetuses of mothers administered various TCB congeners possessed 1,2,4,5-TCB to the greatest extent. The residue profile for 1,2,4,5-TCB in both mothers and fetuses closely resembled the profile obtained with hexachlorobenzene (Villeneuve and Hierlihy, '75) and in general, levels, depending on the tissue and dose, were the same or as much as ten times higher than those reported for pentachlorobenzene (Villeneuve and Khera, '75). It was concluded from the data of both the visceral and skeleton examinations that no teratological potential was evident for the tetrachlorobenzene isomers used in this study. Furthermore, of the three congeners tested, 1,2,4,5-TCB leads to greater toxicity and permeability across the placenta as well as a greater presence in tissue, most likely because of the chlorine positioning.

ACKNOWLEDGMENTS

We are grateful for the help provided by Dr. I.A. Marino during necropsies and the technical assistance of N. Beament, M. Beaudette, B. Reed, J. Kelly, V. Secours, A. Yagminas, A. Viau, and R. Valli.

LITERATURE CITED

Ariyoshi, T., K. Ideguchi, K. Iwasaki, and M. Arakaki (1975a) Relationship between chemical structure and activity. I. Effects of the number of chlorine atoms in chlorinated benzenes on the components of drug-metabolizing system and the hepatic constituents. Chem. Pharm. Bull., *23*:817–823.

Ariyoshi, T., K. Ideguchi, K. Iwasaki, and M. Arakaki (1975b) Relationship between chemical structure and activity. II. Influences of isomers in dichlorobenzene, trichlorobenzene on the activities of drug-metabolizing enzymes. Chem. Pharm. Bull., *23*:824–830.

Becking, G.C. (1973) Vitamin A status and hepatic drug metabolism in the rat. Can. J. Physiol. Pharmacol., *51*:6–11.

Braun, W.H., L.H. Sung, D.G. Keyes, and R.J. Kociba (1978) Pharmacokinetic and toxicological evaluation of dogs fed 1,2,4,5-tetrachlorobenzene in the diet for two years. J. Toxicol. Environ. Health, *4*:727–738.

Cochin, J., and J. Axelrod (1959) Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. J. Pharmacol. Exp. Therap., *125*:105–115.

Conney, A.H. (1967) Pharmacological implication of microsomal enzyme induction. Pharmacol. Rev., *19*:317–366.

Environmental Protection Agency (1980) Assessment of testing needs: chlorinated benzenes. July 1983 EPA-560/11-80-014.

Fouts, J.R. (1963) Factors influencing the metabolism of drugs in liver microsomes. Ann. N.Y. Acad. Sci., *104*:875–880.

Gornall, A.G., C.J. Bardawill, and M.M. David (1948) Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., *177*:751–755.

Hallett, D.J., K.S. Khera, D.R. Stoltz, I. Chu, D.C. Villeneuve, and J. Trivett (1978) Photomirex: synthesis and assessment of acute toxicity, tissue distribution and mutagenicity. J. Agric. Food Chem., *26*:388–391.

Konasewich, D., W. Traversy, and H. Zar (1978) Great Lakes Water Quality Board Appendix E. Status report on organic and heavy metal contaminants in the Lakes Erie, Michigan, Huron, and Superior Basins, Windsor, Ontario, July.

Morita, M., S. Mimura, G. Ohi, H. Yagu, and T. Nishizawa (1975) A systematic determination of chlorinated benzenes in human adipose tissue. Environ. Poll., *9*:175–179.

Veith, G.D., D.W. Kuehl, E.D. Leonard, F.A. Puglisi, and A.E. Lemke (1979) Polychlorinated biphenyls and other organic chemical residues in fish from major watersheds of the United States, 1976. Pest. Monit. J., *13*:1–11.

Villeneuve, D.C., V.E. Valli, I. Chu, V. Secours, L. Ritter, and G.C. Becking (1979) Ninety-day toxicity of photomirex in the male rat. Toxicology, *12*:235–250.

Villeneuve, D.C., and S.L. Hierlihy (1975) Placental transfer of hexachlorobenzene in the rat. Bull. Environ. Contamin. Toxicol., *13*:489–491.

Villeneuve, D.C., and K.S. Khera (1975) Placental transfer of halogenated benzenes (pentachloro-, pentachloronitro-, and hexabromo-) in rats. Env. Physiol. Biochem., *5*:328–331.