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TRICHLOROBENZENES: RESULTS OF A THIRTEEN WEEK FEEDING STUDY IN THE RAT

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ABSTRACT

Trichlorobenzenes (TRCBs) are industrial chemicals and environmental pollutants found in Great Lakes fish. The present study was carried out to provide information on the toxic effects of these chemicals in mammals. Groups of male and female weanling rats were fed diets containing TRCB isomers at 1, 10, 100 or 1000 ppm for 13 weeks. The group of males fed 1000 ppm 1,2,3-TRCB diet had reduced weight gain. No other clinical signs of toxicity were observed. Increased relative liver and kidney weights occurred in the highest dose groups of males for all 3 TRCBs. Of the 3 isomers, only 1,2,4-TRCB at 1000 ppm caused increases in hepatic aminopyrine demethylase and aniline hydroxylase activities in male rats, and aminopyrine demethylase in females. Serum biochemical and hematological parameters measured were not affected. All

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three TRCBs produced histological changes of a moderate degree in the liver and thyroid of male rats at 1,000 ppm. Only 1,3,5-TRCB elicited moderate renal changes in male rats fed 1,000 ppm. Microscopic changes in the females were generally milder than those of the corresponding
males. Based on these data it was concluded that the non-observable adverse effect levels for the three TRCBs were 100 ppm in the diet, or 7.6 ~ 7.8 mg/kg b.w./day depending on the dietary consumption.

INTRODUCTION

Trichlorobenzenes (TRCBs) are industrial chemicals that are used as chemical intermediates in the synthesis of herbicides. These chemicals are also used as dye carriers and as a dielectrical fluid in electrical insulators and transformers[1]. Trichlorobenzenes have been detected in Great Lakes fish, drinking water and waste-water effluents around ng/L levels[2,3]. Because of the wide use of these chemicals together with subsequent release into the environment, concern has been raised over the potential health hazard to humans associated with occupational and environmental exposure. Previous studies have demonstrated that 1,2,4-TRCB caused biochemical changes and hepatomegaly in rats, dogs[4] and rabbits[5]. Metabolism studies in the monkey and rat have shown that 1,2,4-TRCB is biotransformed to a trichlorophenol, a cysteine conjugate and dihydrodiol derivatives[6]. A review of the literature reveals a lack of adequate toxicity data to permit the assessment of health risks in humans exposed to these chemicals. The present study was designed to investigate the toxic effects in the rats treated with trichlorobenzenes in the diet for 90 days.

MATERIALS AND METHODS

1,2,3-, 1,2,4- and 1,3,5-Trichlorobenzene were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). 1,2,3- and 1,3,5-TRCB were purified by recrystallization in hexanes to a purity of >99%. The 1,2,4-isomer, which had a purity of >99%, was used without further purification. The purities of these compounds were confirmed by thin layer chromatography and gas chromatography-mass spectrometry. All other chemicals and solvents were of reagent grade obtained commercially.

Preparation of Diet

The diet consumed by the control groups was prepared by blending thoroughly ground cubes (Ralston Purina) with corn oil (Mazola, 4% w/w). The test diets were made by mixing ground cubes with corn oil solutions containing appropriate amounts of test chemicals to give dietary levels of 1, 10, 100 or 1,000 ppm. Fresh diets were made every fourth week throughout the study, and kept in air-tight steel containers. Selection of dose levels was based on a range-finding study in which the LD_{50} of the three TRCB in rats were found to be 1,2,3-TRCB, 1.83 g/kg; 1,2,4-TRCB, 0.88 g/kg and 1,3,5-TRCB, 2.1 g/kg.

Animal Treatment

Weanling Sprague-Dawley rats (130 males and 130 females), obtained from Charles River, Montreal, were divided into 13 groups of 10 animals per group of each sex. The animals were housed individually in stainless-steel mesh cages and acclimatized to laboratory conditions for one week before initiation of the study.

The room temperature and relative humidity were kept at 22-24°C and 40-60% respectively. A 12 hr alternated light/dark cycle was maintained. The diet that each group of animals of both sexes received is as follows:

Group 1	Control (4% corn oil in ground cubes)
Group 2	1,2,4-Trichlorobenzene (TRCB) 1 ppm in ground cubes
Group 3	1,2,4-TRCB 10 ppm
Group 4	1,2,4-TRCB 100 ppm
Group 5	1,2,4-TRCB 1000 ppm
Group 6	1,2,3-TRCB 1 ppm
Group 7	1,2,3-TRCB 10 ppm
Group 8	1,2,3-TRCB 100 ppm
Group 9	1,2,3-TRCB 1000 ppm
Group 10	1,3,5-TRCB 1 ppm
Group 11	1,3,5-TRCB 10 ppm
Group 12	1,3,5-TRCB 100 ppm
Group 13	1,3,5-TRCB 1000 ppm

Body weight was determined weekly on all animals. In order to avoid possible loss of test chemical by volatility, the diet left in the feeder was replaced with fresh ones on a weekly basis. Food consumption was recorded at Weeks 1, 4, 8 and 12 of study on 5 animals per group of both sexes. Urinalysis (pH, protein and nitrite) was carried out on 5 animals per group per sex at Weeks

4, 8 and 12 of study. Clinical examinations were performed on all animals on a daily basis.

At the end of the 13 week feeding period all animals were anesthetized to surgical level with ether, and exsanguinated via the abdominal aorta. All animals were examined grossly at the time of necropsy. The brain, heart, liver, spleen, and kidneys were excised and weighed. Blood samples collected at necropsy were analyzed for the following parameters: hemoglobin, packed cell volume, erythrocyte count (Baker Model 7000), total and differential leukocyte counts, platelet count, and prothrombin time. Mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin values were calculated. Serum biochemical profiles were determined in a Technicon microanalyzer (Model 12/60) and included sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase, aspartate aminotransferase, total protein, calcium, cholesterol, glucose, uric acid, and lactic dehydrogenase.

Hepatic microsomal aniline hydroxylase (AH)[7] and aminopyrine demethylase (APDM)[8] activities were determined based on the previously reported methods adapted to automated instruments. Liver protein content was determined by the biuret method[9]. A section of femoral bone marrow was aspirated, spread on the slide from which thin films were made, and stained with May-Grünwald-Giemsa stain for cytological evaluation. The following tissues were taken and fixed in 10% buffered formalin (pH 7.4) for routine histological examination: eye, optic nerve, spinal cord, skin, tongue, brain, pituitary, liver, adrenal, thyroid, parathyroid,

thymus, lungs, trachea, bronchi, thoracic aorta, esophagus, gastric cardia, fundus and pylorus, duodenum, jejunum, ileum, pancreas, colon, cecum, kidneys, spleen, bone marrow, mesenteric and mediastinal lymph nodes, skeletal muscle, ovaries, uterus, vagina or testes, prostate, epididymis, sciatic nerve, urinary bladder, salivary gland, mammary gland, and heart. Potential fatty change in the liver was determined in frozen sections as previously described[10].

Sections of liver and perirenal fat were excised and kept at -70°C pending residue analysis using a gas chromatographic method as described previously[10]. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test to indicate the groups which were significantly different from the control ($P \leq 0.05$)[11].

RESULTS

Clinical Observation

Clinical examination of animals revealed no signs of toxicity other than reduced weight gain of some groups. One male rat from the control group died without any apparent cause during the first week. A female rat in the 1,000 ppm 1,3,5-TRCB group died during the 4th week. The cause of death was unknown but did not appear to be treatment-related. The remaining animals survived until the termination of the 13 week feeding period.

Weight Gain and Food Consumption

There was a general trend towards lower weight gain in the high dose groups of treated males but statistically significant

growth suppression was only observed in groups fed 1,2,3-TRCB at 10 or 1,000 ppm. The weight gain and food consumption data and the amount of test compounds ingested by male rats are presented in Table 1. There was no decrease in growth rate or food consumption in female rats. The approximate amount of test compounds ingested by the females, expressed in the form of mg/kg b.w./day are presented below:

Gross Changes

. Fatty liver was observed in some of the male rats but the incidence was of a sporadic nature, occurring in both control and treated groups. One male rat receiving 1000 ppm 1,2,4-TRCB diet had nephrosis. No gross changes were observed in female rats.

Organ Weights

Organ weight data are presented in Table 3. The liver/body weight ratios of males receiving the highest dose of 3 TRCBs were significantly greater than those of the control group. The wet kidney weights as well as kidney/body weight ratios of males receiving 1000 ppm 1,2,4-TRCB; 10 ppm or 100 ppm 1,3,5-TRCB diet were higher compared to control values. The kidney/body weight ratios of the groups of males fed diet containing 1, 10 or 1000 ppm 1,2,3-TRCB or 1000 ppm 1,3,5-TRCB were also significantly greater than the control.

Biochemical Changes

None of the serum biochemical constituents were affected by exposure to TRCBs up to and including 1000 ppm in diet; these

TABLE 1 - Food Consumption and Weight Gain of Male Rats Fed TRCB

Treatment (ppm in the diet)	Food Consumption (g/rat)	Initial Weight (g)	Weight Gain (g)	Amount of[a] Chemical Ingested (mg/kg b.w./day)
Control	25 ± 1.1(5)[b]	85 ± 8(9)	471 ± 54(9)	0
1,2,4-TRCB				
1	23 ± 0.7(5)	85 ± 5	453 ± 27	0.07
10	25 ± 1.2(5)	80 ± 5	474 ± 47	0.78
100	25 ± 1.1(5)	85 ± 10	472 ± 42	7.8
1000	25 ± 2.2(5)	84 ± 10	444 ± 33	82
1,2,3-TRCB				
1	24 ± 1.8(5)	80 ± 5	447 ± 37	0.08
10	23 ± 1.4(5)	82 ± 6	425 ± 43*	0.78
100	24 ± 2.5(5)	84 ± 6	458 ± 47	7.6
1000	23 ± 0.8(5)	80 ± 4	423 ± 42*	78
1,3,5-TRCB				
1	26 ± 1.4(5)	87 ± 9	482 ± 51	0.08
10	27 ± 0.5(5)	89 ± 6	490 ± 34	0.81
100	24 ± 0.9(5)	85 ± 5	450 ± 60	7.7
1000	26 ± 1.8(5)	84 ± 6	450 ± 38	82

[a] The amount of TRCB ingested is an approximate figure derived from the following formula:

$$\frac{\text{food consumption (g) x dietary concentration (ppm)}}{\text{average body weight}}$$

[b] Unless otherwise given in parentheses results represent mean ± S.D. derived from 10 animals. The food consumption was determined in 5 animals per group for all groups.

Table 2 Amount of Chemical Ingested by Female Rats

Compound	mg/kg b.w./day		Compound	mg/kg b.w./day	
1,2,4-TRCB	1 (ppm)	0.11	1,3,5-TRCB	1 (ppm)	0.13
	10	1.4		10	1.5
	100	15		100	17
	1000	101		1000	146
1,2,3-TRCB	1	0.13			
	10	1.3			
	100	12			
	1000	113			

included sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase, aspartate aminotransferase, total protein, calcium, cholesterol, glucose, uric acid and lactate dehydrogenase.

Measurements of hepatic mixed function oxidase activities revealed that 1,2,4-TRCB at the 1000 ppm level caused a significant elevation in AH and APDM activities in males, and APDM activity in females.

AH:	male	μmole PAP/hr/mg protein
	Control:	8.0 ± 3.4
	Treated:	15.6 ± 6.2
APDM:	male	μmole HCHO/hr/mg protein
	Control:	23 ± 3.6
	Treated:	53 ± 19
	female	μmole HCHO/hr/mg protein
	Control:	24 ± 3.1
	Treated:	41 ± 7.4.

The remaining 2 TRCB isomers at the dose levels studied had no effects on these hepatic microsomal enzymes.

TABLE 3 - Organ Weights of Male Rats Fed Diet Containing TRCB (mean ± S.D.)

Treatment (ppm in the diet)	n[a]	Liver		Kidney	
		Wet Weight (g)	Liver/b.w. ratio (% of b.w.)	Wet Weight (g)	Kidney/b.w. ratio (% of b.w.)
Control	9	19.6 ± 2.7	3.5 ± 0.30	1.56 ± 0.20	0.28 ± 0.02
1,2,4-TRCB					
1	10	17.8 ± 2.5	3.3 ± 0.43	1.57 ± 0.11	0.29 ± 0.02
10	10	20.5 ± 2.3	3.7 ± 0.29	1.68 ± 0.18	0.30 ± 0.04
100	10	20.8 ± 2.0	3.7 ± 0.20	1.72 ± 0.10	0.31 ± 0.02
1000	10	22.2 ± 1.5	4.2 ± 0.22*	2.04 ± 0.44*	0.38 ± 0.06*
1,2,3-TRCB					
1	10	18.8 ± 1.7	3.6 ± 0.22	1.68 ± 0.10	0.32 ± 0.03*
10	10	19.3 ± 2.5	3.8 ± 0.29	1.64 ± 0.14	0.32 ± 0.02*
100	10	19.4 ± 3.1	3.6 ± 0.31	1.60 ± 0.20	0.29 ± 0.02
1000	10	20.1 ± 2.5	4.0 ± 3.4*	1.72 ± 0.25	0.34 ± 0.04*
1,3,5-TRCB					
1	10	20.5 ± 2..	3.6 ± 0.24	1.75 ± 0.16	0.31 ± 0.03
10	10	21.8 ± 1.9	3.8 ± 0.21	1.87 ± 0.27*	0.32 ± 0.04*
100	10	20.1 ± 4.2	3.7 ± 0.41	1.85 ± 0.22*	0.35 ± 0.03*
1000	10	21.0 ± 3.4	3.9 ± 0.60*	1.69 ± 0.17	0.32 ± 0.04*

[a] Number of animals.

* Significantly different from the control ($P < 0.05$).

Hematological Changes

Treatment with 3 TRCBs had no effects on any hematological parameters monitored in this study.

Histopathology

The liver, thyroid and kidney were the organs which had treatment-related changes. The changes were qualitatively similar for all 3 TRCB isomers, and for the most part, were mild in nature and judged to be significant only at the highest dose level. In general, morphological alterations in males were more severe than those of females.

Microscopic changes in the livers of most treated groups consisted of a mild to moderate increase in cytoplasmic volume and anisokaryosis of hepatocytes observed mostly in perivenous and midzone areas. Animals treated with 1000 ppm 1,2,4-TRCB had marked changes characterized by aggregated basophilia as well as widespread midzonal vacuolation due to fatty infiltration. The 1,2,3- and 1,3,5-isomers, showed the same changes but were milder.

Changes in the thyroid were characterized by reduction in follicular size, increased epithelial height from flattened cuboidal cells to columnar shape; and reduced colloid density. Depending on the dose, the severity of these changes varied from minimal in the low dose groups to mild and moderate changes in the high dose groups.

Of the three isomers tested, only 1,3,5-TRCB resulted in mild to moderate renal changes in the convoluted tubules. The changes were characterized by eosinophilic inclusions, enlargement and

anisokaryosis of the epithelial lining cells and hyperplasia of renal tubular epithelial cells. The renal changes for most groups were considered to be mild, and only those changes associated with the 1000 ppm dose level were considered to be biologically significant.

Urinalysis

No significant treatment-related changes were observed in any of the urinary parameters monitored.

Residue Analysis

Table 4 presents the residue data in fat and liver of rats. There was a dose-dependent accumulation in both fat and liver in the following order:

1,3,5-TRCB > 1,2,4-TRCB > 1,2,3-TRCB.

The levels of TRCB in fat were one order of magnitude higher than those found in liver.

DISCUSSION

Treatment with TRCBs resulted in growth suppression, increased organ weight, hepatic microsomal enzyme induction and mild histological changes; most of these only occurred in the male rats exposed to the highest dose of TRCBs. The growth suppression observed in the 10 and 1,000 ppm 1,2,3-TRCB but not in the 100 ppm group would indicate that the effect at 10 ppm is probably an incidental finding, since it is not clearly dose-dependent.

TABLE 4 - Trichlorobenzene Residues (ppm) in Liver and Fat[a]

Treatment (ppm in the diet)	Males		Female	
	Liver	Fat	Liver	Fat
Control	ND[b]	ND	ND	ND
1,2,4-TRCB				
1	ND	ND	ND	ND
10	ND	0.37 ± 0.12	ND	0.26 ± 0.03
100	0.25 ± 0.07	3.5 ± 1.1	0.1 ± 0.03	2.7 ± 0.39
1000	2.5 ± 0.71	36 ± 12	1.0 ± 0.83	19 ± 2.7
1,2,3-TRCB				
1	ND	ND	ND	ND
10	ND	0.1 ± 0.04	ND	ND
100	0.24 ± 0.07	1.2 ± 0.31	ND	0.63 ± 0.15
1000	1.4 ± 0.55	15.5 ± 7.4	0.73 ± 0.37	7.8 ± 2.8
1,3,5-TRCB				
1	ND	0.21 ± 0.07	ND	0.1 ± 0.02
10	ND	1.3 ± 0.26	ND	0.43 ± 0.11
100	0.37 ± 0.11	7.3 ± 2.1	0.14 ± 0.03	4.0 ± 0.86
1000	4.3 ± 1.4	76 ± 13	1.9 ± 0.86	49 ± 17

[a] Data represent mean ± S.D. obtained from 5 animals

[b] Non-detectable; the limit of detection is 0.01 ppm.

However there is a general trend towards a lower weight gain in the 1000 ppm groups of males.

In our study, dietary exposure of rats to 3 TRCBs at the 1,000 ppm level resulted in increases of relative liver and kidney weights. Similar findings have been documented by Kociba et al (1981)[4] who observed that rats exposed to 100 ppm of 1,2,4-TRCB by inhalation, 7 h/day for 30 days had increased liver and kidney weights.

Although the relative kidney weights of the males exposed to 1, 10 and 1,000 ppm 1,2,3-TRCB or 1000 ppm 1,2,4-TRCB were statistically higher than those of the controls (Table 3), histopathological examination of this organ failed to reveal any abnormal changes, neither did the urinalysis. However, the increased renal weight at 10, 100 and 1000 ppm 1,3,5-TRCB was accompanied by histological changes.

Of the 3 TRCBs examined in the present study, only the 1,2,4-isomer resulted in elevated hepatic microsomal AH and APDM activities. These data are consistent with those of Goldstein et al (1982)[12] who reported an increase in rat hepatic microsomal APDM activity following administration of chlorinated benzenes including 1,2,4-TRCB at 250 mg/kg b.w./day for 7 days. The hepatic microsomal enzyme induction observed in the present study is consistent with the morphological changes in the liver characterized by increased eosinophilia and cytoplasmic volume. Ariyoshi et al (1975 a,b)[13,14] have shown that 1,3,5-TRCB is capable of inducing hepatic microsomal AH activity in rat at 250 mg/kg b.w. Thus the

failure to observe increased AH activity in our study may be attributed to the lower dose used (82 mg/kg b.w./day, Table 1).

Tissue residue data in the present study revealed that 1,3,5-TRCB residues accumulated to a higher level in fat and liver than those of the other two isomers. These data confirmed the results of our earlier pharmacokinetic study which demonstrated that tissue ^{14}C-contents was the highest for rats dosed orally with 1,3,5-TRCB (Chu et al, 1987)[15].

Histopathological changes for the most part were mild in nature. Only those produced in the liver and thyroid at 1000 ppm of 3 TRCBs as well as those in the kidney by 1000 ppm 1,3,5-TRCB were considered to be biologically significant. At these dose levels, some functional changes were also observed.

In the present study the effects observed for TRCB isomers can be summarized as follows:

1,2,4-TRCB: Significant increases in liver and kidney weights, and microsomal enzyme activities at 1,000 ppm; mild to moderate histological changes in the liver and thyroid at 1,000 ppm.

1,2,3-TRCB: Growth suppression at 1000 ppm; mild to moderate morphological changes in the liver and thyroid at 1,000 ppm.

1,3,5-TRCB: Moderate histological changes in the kidney, liver and thyroid at 1,000 ppm; increased relative liver and kidney weights at 1,000 ppm.

Based on the data summarized above, we conclude that the non-observable adverse effect levels are 100 ppm for all three TRCBs or 7.8 mg/kg b.w./day (1,2,4-), 7.7 mg/kg b.w./day (1,2,3-) or 7.6 mg/kg b.w./day (1,3,5-) depending on the dietary consumption.

The highest TRCB (1,2,4-) level found in lake trout is 5 ppb[3]. Assuming the average fish consumption is 16.6 g/day/person[16], this would give rise to an estimated daily intake of 83 ng TRCB/person/day for a person of 60 kg b.w. or 1.4 ng TRCB/kg b.w./day. Since the non-observable adverse effect level has been found to be 7.8 mg/kg b.w./day (1,2,4-), there exists a 5.6×10^6 times safety factor between the potential human exposure and the effect level in the rat.

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8.7 Developmental and Reproductive Effects

The results of studies concerning the embryotoxicity, fetotoxicity, teratogenicity, and reproductive effects of the chlorobenzenes are presented in Table 24. Effect levels derived in these studies for both mothers and offspring are also presented in this table. In contrast to the lack of information on acute, short-term, long-term, and carcinogenic effects, comparatively more data are available on the potential embryotoxic, fetotoxic, and teratogenic effects of the higher chlorinated benzenes. Furthermore, the TCBs, TeCBs, and PeCB have been better studied in this regard than MCB and the isomers of DCB.

There has been no evidence in any of the studies conducted to date that the chlorobenzenes (mono- to penta-) are teratogenic in animal species. Some relatively minor embryotoxic and fetotoxic effects have been observed for the lower chlorinated benzenes (MCB and DCBs), but only at dose levels that were toxic for the mother. For example, there was a slight delay in skeletal development (ossification) in the fetuses of pregnant rats exposed to 2864 mg MCB/m^3, a dose that induced decreases in body weight gain in the mothers (John et al., 1984). Similarly, the ossification of cervical vertebrae in fetuses of pregnant rats exposed to 2400 mg 1,2-DCB/m^3 was delayed; this dose also induced decreases in body weight gain and food consumption in the mothers (Hayes et al., 1985). However, in both of these studies, there was no convincing evidence of embryotoxic, fetotoxic, or teratogenic effects in rabbits exposed to MCB or 1,2-DCB via inhalation, even at dose levels that were maternally toxic (John et al., 1984; Hayes et al., 1985). In fetuses of pregnant rabbits exposed to 4720 mg 1,4-DCB/m^3, there was an increase in the incidence of retro-oesophageal right subclavian artery, a minor variation in the circulatory system that is often observed in control litters; at this dose, the maternal body weight gain was also decreased (Hayes et al., 1985). In an additional study, Giavini et al. (1986)

administered 1,4-DCB, in corn oil, by gavage, at doses of 250, 500, 750, or 1000 mg/kg body weight per day, between days 6 and 15 of gestation. Effects were noted only at doses greater than 500 mg/kg per day. These included an increased frequency of extra ribs, a reduction in fetal weight, and an increase in skeletal abnormalities. These dose levels also induced decreases in body weight gain and food consumption in the mothers.

There is some evidence that the higher chlorinated benzenes (TCBs, TeCBs, PeCB) are embryotoxic or fetotoxic at dose levels that are not maternally toxic. However, available data are not consistent and the toxicities of the various isomers of the TCBs and TeCBs for the mother and fetus vary considerably. For example, the 1,2,4-isomer was the most maternally toxic of the 3 TCB isomers administered by ingestion to pregnant rats in a study conducted by Black et al. (1988); changes in haematological parameters occurred at doses as low as 150 mg/kg per day. At a lower dose (75 mg/kg), there were mild histological changes in the lenses of pups of exposed mothers; however, these changes were not observed at higher doses (150 or 300 mg/kg) and were unlikely, therefore, to be treatment-related. Kitchin & Ebron (1983a) observed growth retardation in the embryos of pregnant rats administered 360 mg 1,2,4-TCB/kg body weight on days 9-13, a dose that caused some lethality, reduced body weight gain, and produced moderate hepatocellular hypertrophy in mothers.

Although less toxic for the mothers than 1,2,4-TCB, the 1,3,5-isomer of trichlorobenzene induced mild changes in the lenses of pups of

pregnant rats administered doses as low as 150 mg/kg body weight by gavage; there was no significant maternal toxicity at this dose (Black et al., 1988). For the 1,2,3-isomer, there were no effects in offspring at any dose level (up to 600 mg/kg body weight), even though a level of 300 mg/kg was toxic to the mothers. The authors of this study did not discuss the significance of the observed histological changes in the lenses (areas of cellular disorientation and disaggregation with ballooning and granular degeneration) of the pups of mothers administered the 1,3,5-isomer, but concluded that there was no evidence that any of the TCB congeners were teratogenic or fetotoxic.

Kacew et al. (1984) reported that the 1,2,4,5-isomer was the most toxic of the TeCBs for both mothers and fetuses, in a study in which all 3 isomers were administered to pregnant rats by gavage (death of 9/10 animals at 200 mg/kg and increase in serum cholesterol at 50 mg/kg body weight). The toxicity was well correlated with the greater accumulation of 1,2,4,5-TeCB in maternal and fetal tissues. There was a decrease in the number of live fetuses in pregnant rats administered 50 mg 1,2,4,5-TeCB/kg body weight, a dose that induced minor changes (increases in serum cholesterol) in exposed mothers.

Table 24. Developmental and reproductive studies on chlorobenzenes

Compound; Reference	Species[a]	Dose[b,c]	Results[d]	Effect Levels[e]
1,2,3-TCB 1,2,4-TCB 1,3,5-TCB (99.5%) Ruddick et al. (1983); Black et al. (1988)	Sprague-Dawley rat (pregnant); 14/group	1,2,4-TCB: 0, 75, 150, or 300 mg/kg per day; 1,2,3-TCB and 1,3,5-TCB: 0, 150, 300 or 600 mg/kg per day on days 6-15 of gestation; gavage in corn oil	maternal toxicity - reduced body weight gain at 600 mg/kg (1,3,5-TCB), increased liver weight at 600 mg/kg (1,2,3- and 1,3,5-TCB) and 300 mg/kg (1,2,4-TCB), decreased haemoglobin levels and haematocrit, generally at highest dose (all 3 isomers), decreased RBC at 300 mg/kg (1,2,3-TCB), 150 and 300 mg/kg (1,2,4-TCB), and 150 and	1,2,3-TCB: 300 mg/kg per day (F); *600 mg/kg per day (O) (NOEL) 1,2,4-TCB: