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Long-Term Toxicity of Octachlorostyrene in the Rat

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Long-Term Toxicity of Octachlorostyrene in the Rat. CHU, I., VILLENEUVE, D. C., SECOURS, V. E., VALLI, V. E., LEESON, S., AND SHEN, S. Y. (1986). *Fundam. Appl. Toxicol.* **6**, 69–77. This study was designed to investigate the toxic effects produced by the long-term exposure to octachlorostyrene (OCS), a demonstrated environmental contaminant in the Great Lakes region of North America and the Norwegian Coast in Europe. Groups of 20 male and 20 female rats were administered OCS in diets at 0.005, 0.05, 0.5, 5.0, or 50 ppm for 12 months. Weight gain and food consumption were not affected. Increased liver weights were observed in the groups fed the highest dose of OCS. Hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities were induced in male rats fed 5.0 ppm OCS and higher and in female rats fed 50 ppm of the chemical. Elevated serum cholesterol levels were seen in rats of both sexes fed the highest dose. Treatment-related histological changes occurred in the thyroid, liver, and kidney of rats. A dose-dependent accumulation of OCS in the fat and liver of the rats was found. Based on the data presented, it was concluded that the no adverse effect level of OCS was 0.5 ppm. © 1986 Society of Toxicology.

Octachlorostyrene (OCS, Fig. 1) is an environmental pollutant that has been identified in Great Lakes fish (Kuehl *et al.*, 1976) and herons (Reichel *et al.*, 1977) at concentrations of 0.01–0.43 ppm. This chemical has also been found in fish (Ofstad *et al.*, 1978) and at concentrations up to 5.0 ppb in human serum samples (Lunde and Bjorseth, 1977) in Norway. There is no commercial application of OCS and the source of contamination is not known, but it is believed to be a by-product in industrial processes involving reactions of carbon and chlorine at high temperature. Because of its wide presence as an environmental contaminant, concern has been raised regarding possible biological effects upon humans or animals from its toxicity and bioaccumulation. Previous acute and subchronic studies have demonstrated that OCS produced serum biochemical changes, hepatic microsomal enzyme induction, and histological changes in the liver, thyroid, and kidney of rats (Chu *et al.*, 1982a, 1984; Holme and Dybing, 1983). In a metabolism study in rats it was shown that most of the administered OCS was stored in the fatty tissues whilst a small fraction of the dose was excreted in feces as pentachlorophenyldichloroacetic acid (Chu *et al.*, 1982b). The purpose of the present study was to determine the effects of long-term feeding of OCS to rats.

METHODS

Octachlorostyrene was synthesized based on a procedure described previously (Bieniek and Korte, 1978). The procedure involved chlorination of 2,6-dichlorostyrene with chlorine gas, followed by perchlorination with antimony pentachloride. The product obtained was purified by column chromatography with silica gel and fractional crystallization in 95% ethanol to a purity of greater than 99% as indicated by TLC and GC. The melting point was found to be 100.5–101°C (Lit. m.p. 99–100°C, Ruetman, 1973).

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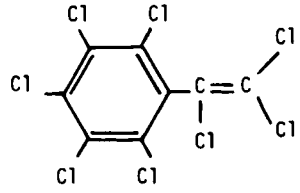

FIG. 1. Structure of octachlorostyrene.

The identity of the compound was confirmed by GC/MS (M^+ at m/e 376) (Finnigan Instrument, Model 4000, data system 6000). All other chemicals used were of commonly available reagent grade.

Groups of 20 male and 20 female weanling Sprague–Dawley rats (Charles River, Boston) were fed pelleted diets (Master Fox, Purina-Ralston) containing 0, 0.005, 0.05, 0.5, 5.0, or 50 ppm OCS for 12 months. Corn oil (4% of total diet) was used as a vehicle to dissolve the test chemical and to facilitate its incorporation into the diet. The control groups received diets containing 4% corn oil. The respective diets were then pelleted and offered to the animals. All animals were housed individually in stainless-steel mesh cages with free access to food and water. Clinical observations were made daily; body weight gain and food consumption were measured weekly. At the termination of the feeding period all animals were lightly anesthetized 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. Serum biochemical determinations were made using a Technicon microanalyzer, Model 12/60 and included sodium, potassium, inorganic phosphate, total bilirubin, alkaline phosphatase, glutamic oxalacetic transaminase (GOT), total protein, calcium, cholesterol, glucose, uric acid, and lactic dehydrogenase. Sorbitol dehydrogenase (SDH) activity was determined according to an automated method as previously described (Yagminas and Villeneuve, 1977). Hepatic microsomal enzyme activities were determined by an automated method developed in our laboratories and included aniline hydroxylase (AH; Fouts, 1963), and aminopyrine demethylase activities (APDM; Cochin and Axelrod, 1959). Liver porphyrin concentrations were measured according to the method described by Schwartz and Wikoff (1952). A selection of tissues was taken and fixed in 10% buffered Formalin (pH 7.4) for routine histological examination. The tissues examined histologically included brain, pituitary, liver, adrenal, thyroid, parathyroid, thymus, lungs, trachea, bronchi, thoracic aorta, esophagus, gastric cardia, fundus and pylorus, duodenum, jejunum, ileum, pancreas, colon, kidney, spleen, bone marrow, mesenteric and mediastinal lymph nodes, testes, epididymis, skeletal muscle, and heart. Fatty change in liver was determined in frozen sections as previously described (Villeneuve *et al.*, 1979). The following hematological parameters were determined from blood samples taken from each animal at the necropsy: hemoglobin, packed cell volume (PCV), red blood cells count, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, and total (Baker 7000 cell counter) and differential count of white blood cells. In addition, bone marrow was aspirated from the femur in order to prepare smears for differential counts (Villeneuve *et al.*, 1979).

Each batch of pelleted diets was analyzed by GC to ensure that the test animals were fed intended levels of OCS. Residues of OCS in diets, liver, and adipose tissue were analyzed in a similar manner described previously (Villeneuve *et al.*, 1979). Statistical analysis of data was carried out using one-way analysis of variance. Where significant difference was noted the data were further treated with the Duncan's multiple range test (Nie *et al.*, 1977) in order to indicate specific groups that were significantly different from the controls.

RESULTS

Clinical Observation

No clinical signs of toxicity were observed. OCS treatment had no effects on the weight gain and dietary consumption of female rats. The male rats fed 0.005, 0.5, and 50 ppm of the test chemical showed increased body weight, but not food consumption (Table 1). Seven male rats among the various groups died before termination of the study. The cause of death in most cases was unknown and it did not appear to be treatment related (one animal each from the control, 0.05 and 0.5 ppm groups; two animals each from 0.005 and 5.0 ppm groups). In the 5.0 ppm males, one animal had multiple foci of papillary carcinoma in the lungs and probably died of respiratory failure, while the other had immunoblastic sarcoma of the spleen. One female rat from the control group probably died of thymic lymphoma. Two females, one each from 0.005 and 5.0 ppm groups died spontaneously. All of the deaths occurred between 6 to 12 months of experiment. Based on the body weight and dietary consumption data, the OCS ingested ranged from 0.0003 to 3.1 mg/kg body wt/day and 0.0004 to 4.4 mg/kg body wt/day for the males and females, respectively (Table 1).

TABLE 1

BODY WEIGHT AND FOOD CONSUMPTION OF RATS FED OCTACHLOROSTYRENE[a]

Treatment octachlorostyrene (ppm)	Initial weight (g)	Final weight (g)	Food consumption g/animal/day[c]	Approximate amount of chemical ingested (mg/kg body wt/day)
		Male		
Control	156 ± 20	775 ± 99	31 ± 2.5	0
0.005	152 ± 22	857 ± 95[b]	31 ± 2.6	0.0003
0.05	157 ± 25	814 ± 91	30 ± 2.2	0.003
0.5	152 ± 28	829 ± 142[b]	30 ± 4.2	0.031
5.0	156 ± 27	799 ± 79	29 ± 2.9	0.31
50	167 ± 29	842 ± 93[b]	31 ± 2.4	3.1
		Female		
Control	111 ± 14	360 ± 71	20 ± 2.5	0
0.005	116 ± 16	362 ± 65	20 ± 2.7	0.0004
0.05	114 ± 16	366 ± 52	20 ± 1.8	0.004
0.5	115 ± 17	368 ± 53	20 ± 2.4	0.042
5.0	115 ± 17	378 ± 47	20 ± 1.8	0.4
50	118 ± 21	380 ± 59	21 ± 2.1	4.4

[a] Results represent mean ± SD obtained from 18 to 20 animals.

[b] Different from controls at the $p \leq 0.05$ level.

[c] The food consumption represents the average values obtained by totalling the individual food intakes at different times and dividing the sum with the number of measurements.

[d] The approximate amount of test chemicals ingested based on mg/kg body wt/day is calculated by: 1000/[(initial wt + final wt)(2 × 1000)] × ppm × food consumption.

Gross Changes

Hepatomegaly was observed in both male and female rats fed the highest dose of OCS. Changes of a sporadic nature included unilateral cataracts, galactoceles, and mammary gland tumours in female rats; enlarged pituitary and adrenal glands, and nephrosis in both sexes. These changes were found in both control and treated animals.

Organ Weights

Increased wet liver and kidney weights were observed in some groups. When these values were expressed as percentage body weight, only the liver weights of male and female rats of the highest dose groups were significantly higher than controls (Table 2).

Biochemical Changes

Of the serum biochemical parameters examined, the level of cholesterol was significantly elevated in female rats receiving the highest dose (control: 109 ± 14; treated: 165 ± 42 mg/dl; $p \leq 0.05$). There was a trend toward higher cholesterol levels in the male rats but this was not statistically significant. Decreased SGOT levels were seen in males fed 0.05 or 0.5 ppm, while decreased alkaline phosphatase was observed in the females in the 50 ppm group. The following biochemical parameters were not affected: calcium, inorganic phosphate, glucose, uric acid, total protein, bilirubin, lactic dehydrogenase activity, sodium, potassium, sorbitol dehydrogenase activity, and liver protein.

Measurement of hepatic microsomal enzymes showed increased aniline hydroxylase

TABLE 2

BIOCHEMICAL CHANGES OF RATS FED OCTACHLOROSTYRENE[a]

Treatment octachlorostyrene (ppm)	Wet liver weight (g)	Liver weight % body wt	Aniline hydroxylase nmol HCHO/mg protein/hr	Aminopyrine demethylase nmol/mg protein/hr
		Male		
Control	23 ± 2.8	3.1 ± 0.24	11.0 ± 3.3 (16)	44 ± 8.7 (16)
0.005	27 ± 3.2[b]	3.2 ± 0.18	9.7 ± 3.7 (15)	40 ± 9.8 (15)
0.05	26 ± 3.8[b]	3.2 ± 0.23	11.3 ± 3.7 (15)	45 ± 11 (14)
0.5	27 ± 5.4[b]	3.2 ± 0.26	10.2 ± 3.6 (14)	42 ± 9.8 (13)
5.0	26 ± 3.9[b]	3.2 ± 0.39	16 ± 5.8 (13)[b]	58 ± 16 (13)[b]
50	30 ± 4.6[b]	3.6 ± 0.54[b]	17 ± 6.6 (14)[b]	63 ± 19 (14)[b]
		Female		
Control	11 ± 2.2	3.1 ± 0.24	13 ± 3.4 (16)	37 ± 7.7 (16)
0.005	12 ± 1.8	3.3 ± 0.38	12 ± 2.3	32 ± 4.2
0.05	12 ± 2.1	3.2 ± 0.34	12 ± 4.0	35 ± 7.0
0.5	11 ± 1.7	3.1 ± 0.46	13 ± 2.4	31 ± 6.6
5.0	12 ± 1.5	3.3 ± 0.26	15 ± 4.5	39 ± 8.1
50	15 ± 2.2[b]	4.1 ± 0.60[b]	21 ± 3.4 (17)[b]	55 ± 11 (17)[b]

[a] Unless otherwise indicated in parentheses, results represent mean ± SD obtained from 18 to 20 animals.
[b] Significantly different from control at the $p \leq 0.05$ level.

and aminopyrine demethylase activities at 5.0 ppm and higher for male rats and at 50 ppm for females (Table 2).

Hematology

The only hematological parameter affected by treatment was the hemoglobin value (control: 13.9 ± 0.5; treated: 13.5 ± 0.7 g%; $p = 0.02$) of the female rats fed 50 ppm OCS. There was a trend toward lower red cell counts and packed cell volume in this group but the change was not statistically significant. However, all hematological parameters determined in the present study still fell within the normal range for Sprague–Dawley rats established in our laboratories.

Histopathology

The liver, thyroid, and kidney were the target organs affected by OCS treatment. Morphological changes were qualitatively similar for both male and female rats, and generally these changes were more severe in the former. The incidence of these changes is presented in Table 3. Dose-dependent increases in the severity and incidence of changes were observed.

At low magnifications the liver architecture had moderate to marked accentuations of zonations which showed increased frequencies at higher dose levels. Hepatocellular nuclei of the treated animals had mild to marked anisokaryosis. Cytoplasmic vacuolation and an increased ground-glass appearance of the cytoplasm in the perivenous and midzone areas were seen in the 0.005–0.5 ppm groups. However, these changes were very mild. At the 5.0 and 50 ppm levels, moderate to marked cytoplasmic vacuolization together with periportal eosinophilia and aggregated basophilia in the periphery of the hepatocytes were observed; these changes were considered to be moderate to severe in nature.

Treatment-related changes were seen in the

TABLE 3

INCIDENCE OF HISTOLOGICAL CHANGES IN RATS TREATED WITH OCTACHLOROSTYRENE FOR 12 MONTHS[a,b]

Treatment octachlorostyrene (ppm)	Liver					Thyroid				Kidney			
	Architecture	Hepatocellular nuclei	Hepatocellular cytoplasm	Bile duct	Interstitium	Architecture	Epithelium	Colloid	Interstitium	Architecture	Glomeruli	Tubules	Interstitium
						Male							
Control	4/20	1/20	6/20	0/20	0/20	4/19	7/19	2/19	0/19	5/19	9/19	4/19	4/19
0.005	8/19	6/19	18/19	1/19	3/19	9/19	15/19	8/19	0/19	8/19	9/19	10/19	9/19
0.05	5/20	19/20	20/20	3/20	1/20	17/20	19/20	16/20	0/20	11/20	15/20	14/20	13/20
0.5	6/19	19/19	19/19	3/19	0/19	12/19	18/19	7/19	0/19	9/19	12/19	12/19	11/19
5.0	17/20	20/20	20/20	2/20	2/20	14/20	19/20	16/20	0/20	7/20	11/20	11/20	8/20
50	19/19	19/19	19/19	7/19	2/19	18/19	19/19	18/19	0/19	14/19	16/19	18/19	14/19
						Female							
Control	3/20	2/20	3/20	2/20	3/20	2/20	4/20	3/20	0/20	2/20	10/20	3/20	3/20
0.005	2/19	5/19	12/19	3/19	1/19	11/19	15/19	9/19	1/19	8/19	14/19	12/19	7/19
0.05	1/20	6/20	10/20	7/20	2/20	15/20	16/20	9/20	0/20	5/20	13/20	12/20	5/20
0.5	6/20	11/20	14/20	7/20	2/20	15/20	20/20	6/20	0/20	4/20	13/20	8/20	6/20
5.0	5/19	12/19	13/19	3/19	0/19	18/19	19/19	9/19	0/19	1/19	14/19	18/19	2/19
50	18/20	20/20	20/20	8/20	1/20	20/20	20/20	18/20	0/20	8/20	16/20	20/20	11/20

[a] Results denote (number of animals showing changes)/(number of animals examined).

[b] Specific changes: Liver: architecture—accentuated zonation. Hepatocellular nuclei–anisokaryosis. Hepatocellular cytoplasm—increased cytoplasmic volume and eosinophilia, aggregated basophilia, cytoplasmic vacuolation. Bile duct—hyperplasia, sclerosis, focal cuffing. Interstitium—lymphoid aggregates, focal injury. Thyroid: architecture—reduced follicular size, angular collapse of follicles. Epithelium—increased epithelial height. Colloid—reduced density. Interstitium—presence of squamous cysts. Kidney: Architecture—focal or multiple foci scarring. Glomerulus—adhesions and/or sclerosis. Tubules—dilations, eosinophilic inclusions, formation of casts. Interstitium—lymphoid reaction, sclerosis.

thyroid and consisted of reduced colloid density, focal collapse of follicles, and increased epithelial height. At the 0.005–0.5 ppm range, very mild reduction in colloid density and scattered collapse of follicles were noted. In animals receiving 5.0 and 50 ppm, moderate reduction in colloid dnsity and thickening of epithelial cells to columnar form were seen. In addition, focal vacuolation and papillary proliferation were seen in the epithelial tissues and were considered to be moderate to severe in nature.

Morphological changes in the kidney were less severe than those found in the liver and thyroid. OCS at levels up to 0.5 ppm produced changes such as multiple adhesions in glomeruli and thickening of the basement membranes. The glomerular changes were considered to be mild in nature. At 5.0 ppm and higher treatment-related changes were observed and consisted of dilation of proximal tubules and increased eosinophilic intracytoplasmic inclusions associated with proteinaceous loss and formation of granular casts.

The incidence of tumors found in various organs and tissues is presented in Table 4. Adenomas of the pituitary glands were common in the control and treated animals. A few skin and mammary tumors were observed in the female rats. There were no apparent treatment-related effects associated with these tumors.

GC analysis showed a dose-dependent accumulation of OCS residue in fat, liver and

TABLE 4

INCIDENCE OF TUMORS IN RATS FED OCTACHLOROSTYRENE[a]

	Dietary treatment (ppm octachlorostyrene)											
	Male						Female					
	Control	0.005	0.05	0.5	5.0	50	Control	0.005	0.05	0.5	5.0	50
Pituitary												
Adenoma	0/19	0/16	1/17	0/18	0/19	0/19	4/20	2/19	1/19	5/20	0/19	5/20
Adrenal												
Cortical adenoma	0/20	0/18	0/20	0/17	0/20	0/16	1/20	0/19	0/20	0/20	1/19	0/20
Medullary adenoma	0/20	1/18	0/20	0/17	0/20	0/16	0/20	0/19	0/20	0/20	0/19	0/20
Skin												
Sarcoma	0/20	1/18	0/19	0/19	0/18	0/17	0/20	0/19	0/20	0/20	0/19	0/20
Fibroma	1/20	0/18	0/19	0/19	0/18	0/17	0/20	0/19	0/20	0/20	1/19	0/20
Mammary gland												
Adenocarcinoma							0/20	1/19	0/20	0/20	1/19	0/20
Fibroadenoma							0/20	1/19	1/20	0/20	0/19	0/20
Spleen												
Immunoblastic sarcoma	0/20	0/20	0/20	0/19	1/20	0/18	0/20	0/19	0/20	0/20	0/20	0/20
Thyroid												
Carcinoma	0/19	0/19	0/20	0/19	1/19	0/19	0/20	0/19	0/20	0/20	0/19	0/20
Thymus												
Thymic lymphoma	0/19	0/19	0/20	0/19	1/19	0/19	1/20	0/19	0/20	0/20	0/19	0/20
Lungs												
Hemangioma	0/20	0/19	0/20	0/19	0/19	0/19	1/20	0/19	0/20	0/20	0/19	0/20
Papillary carcinoma	0/20	0/19	0/20	0/19	1/19	0/19	0/20	0/19	0/20	0/20	0/19	0/20

[a] Values denote (number of animals showing tumors)/(number of animals examined).

TABLE 5

RESIDUES OF OCTACHLOROSTYRENE IN RAT TISSUES[a]

	Tissues					
	Male			Female		
Treatment	Liver	Kidney	Fat	Liver	Kidney	Fat
Control	0.14 ± 0.29	0.07 ± 0.01	2.8 ± 0.69	0.07 ± 0.07	0.07 ± 0.03	1.0 ± 0.41
0.005	0.07 ± 0.04	0.07 ± 0.04	1.4 ± 0.44	0.04 ± 0.01	0.05 ± 0.02	0.75 ± 0.11
0.05	0.17 ± 0.20	0.14 ± 0.02	4.7 ± 1.8	0.12 ± 0.04	0.06 ± 0.03	3.0 ± 0.63
0.5	0.47 ± 0.08	0.40 ± 0.14	17 ± 3.0	0.55 ± 0.11	0.22 ± 0.03	12 ± 2.5
	1.0 ± 1.0[b]		16 ± 8.9[b]	0.36 ± 0.14[b]		7.5 ± 0.76[b]
	0.39 ± 0.1[c]		7.7 ± 1.2[c]	0.42 ± 0.14[c]		10 ± 3.4[c]
5.0	5.9 ± 1.1	4.4 ± 1.4	166 ± 28	4.75 ± 1.22	2.7 ± 0.95	124 ± 16
	3.6 ± 0.90[b]		79 ± 9.8[b]	2.8 ± 1.0[b]		84 ± 53[b]
	3.2 ± 1.4[c]		80 ± 19[c]	3.0 ± 1.1[c]		66 ± 7.5[c]
50	40 ± 8.5	30 ± 10	1587 ± 308	45.3 ± 12	15 ± 1.3	1189 ± 307
	37 ± 9.3[b]		924 ± 686[b]	26 ± 7.5[b]		1020 ± 500[b]
	27 ± 19[c]		682 ± 123[c]	26 ± 6.6[c]		679 ± 203[c]

[a] Results represent mean ± SD ppm (g wet tissues) obtained from five or more rats.

[b,c] 90- and 28-day exposure data from the groups receiving the same doses of the test chemical, these data are purchased here for the purpose of comparison (Chu *et al.*, 1984a).

kidney (Table 5). The levels of OCS in fat were much higher than those in liver and kidney. The residue data from the 28-day and 90-day feeding experiments are also included in the table for the purpose of comparison.

DISCUSSION

The results of the 12-month study presented above are in general agreement with those of previous 28-day (Chu *et al.*, 1982a) and 90-day studies (Chu *et al.*, 1984) in which rats were treated with OCS in the diet at concentrations up to 500 ppm. The effects observed in those studies included hepatomegaly, increased serum cholesterol, hepatic microsomal enzyme induction, and histological changes in the liver, thyroid, and kidney. However, the erythroid cell depression and meyloid hypertrophy reported in the 90-day study were not seen in the long-term experiment (Chu *et al.*, 1984). Reasons for the discrepancy in erythroid depression were not clear but may represent some adaptive changes seen in the 90-day study. Morphological changes in the liver, kidney, and thyroid were similar to those observed in the 28-day and 90-day studies. Changes in the liver consisted of cytoplasmic enlargement, aniksokaryosis and aggregated basophilia. The cytoplasmic enlargement observed was probably due to the proliferation of smooth endoplasmic reticulum and would account for the microsomal enzyme reduction. Parallel to these changes was the increased serum cholesterol levels. It has been reported that cholesterol synthesis and/or metabolism is mediated by an hepatic microsomal enzyme (Kuntzman, 1969). A comparison was made between the rats fed OCS for 3 and 12 months in order to determine if prolonged exposure would result in an increased severity of histological changes in the target organs (Table 6). The thyroid was the only organ which exhibited more severe changes in the 12-month exposure. The dose levels at which these

changes occurred were 5.0 ppm and 50 ppm. At dose levels lower than these, no difference in severity was observed.

All of the tumors observed in the present study were considered to be incidental and not treatment related. Pituitary and adrenal adenomas are common in aging Sprague–Dawley rats (Furth *et al.*, 1976). Similarly, the spontaneous lactation observed was due to increased secretion of prolactin in aging rats (Berg, 1967). It should be recognized that the study time was for 1 year, therefore the inability of OCS to produce tumors should not be interpreted as negative carcinogenic response.

Octachlorostyrene is a compound of very high lipophilicity with a partition coefficient, log P = 6.8–7.7 (Chu, unpublished data). It would be expected that this compound would accumulate at high concentrations in the adipose tissue. Comparing the levels of OCS residue in rat tissues from this and previous studies (Table 5), it would appear that there is some increase after the 90-day exposure. However, due to the variability of data, we were unable to precisely determine whether the levels of OCS plateaued or further increased after the 90-day exposure.

In a chronic study, Grant *et al.* (1974) reported that hexachlorobenzene elicited hepatomegaly at 80 ppm; and increased liver aniline hydroxylase activity at 10 ppm and aminopyrine demethylase activity at 20 ppm. Thus it would appear that the potency as an enzyme inducer of the structurally similar compound, OCS is comparable to that of hexachlorobenzene. On the other hand, unlike hexachlorobenzene, OCS is not porphyrogenic in rats (Strik and Koeman, 1975).

Data from the chronic study indicates that histological evaluation is the most sensitive index of OCS exposure since changes were observed in the target organs at levels between 0.005 and 0.5 ppm. However, these changes are considered to be mild and adaptative in nature. Significant histological changes only

TABLE 6

SEVERITY OF HISTOLOGICAL CHANGES IN THYROIDS OF RATS FED OCS FOR (A) 12 MONTHS AND (B) 90 DAYS[a]

Treatment (ppm OCS)	Architecture		Epithelium		Colloid		Interstitium	
	A	B	A	B	A	B	A	B
				Male rats				
0 (Control)	1.3 ± 0.56	1.3 ± 0.47	1.4 ± 0.50	1.6 ± 0.51	1.1 ± 0.32	1.0 ± 0	1.0 ± 0	1.0 ± 0
0.05	2.4 ± 0.81[b]	1.6 ± 0.76	2.9 ± 1.1[b]	2.1 ± 0.54	2.0 ± 0.69[b]	1.3 ± 0.47[b]	1.0 ± 0	1.0 ± 0
0.5	1.8 ± 0.77	2.1 ± 0.88	2.5 ± 0.69	2.4 ± 0.91	1.4 ± 0.61	1.6 ± 0.74	1.0 ± 0	1.0 ± 0
5.0	2.2 ± 1.2	1.9 ± 0.80	2.8 ± 0.93	2.5 ± 0.74	2.4 ± 0.88[b]	1.6 ± 0.74	1.0 ± 0	1.0 ± 0
50	3.4 ± 1.0[b]	2.2 ± 0.94	4.3 ± 1.4[b]	2.6 ± 0.63	2.8 ± 1.0[b]	1.9 ± 0.70	1.0 ± 0	1.0 ± 0
				Female rats				
0 (Control)	1.1 ± 0.32	1.0 ± 0	1 2 ± 0.42	1.1 ± 0.26	1.2 ± 0.38	1.0 ± 0	1.0 ± 0	1.1 ± 0.52
0.05	2.2 ± 0.83	1.7 ± 0.70	2.4 ± 0.81[b]	1.6 ± 0.74	1.7 ± 0.67[b]	1.0 ± 0	1.0 ± 0	1.0 ± 0
0.5	2.3 ± 0.91	2.4 ± 0.91	2.6 ± 0.61	2.1 ± 0.74	1.4 ± 0.59	1.1 ± 0.26	1.0 ± 0	1.0 ± 0
5.0	3.3 ± 8.7[b]	2.6 ± 0.91	3.3 ± 0.93[b]	2.2 ± 0.86	1.7 ± 0.86[b]	1.1 ± 0.26	1.0 ± 0	1.0 ± 0
50	4.0 ± 0.90[b]	1.7 ± 1.0	4.0 ± 1.1[b]	1.8 ± 0.80	2.6 ± 0.75[b]	1.2 ± 0.43	1.0 ± 0	1.0 ± 0

[a] Histologic gradings are illustrated as follows: 1 is designed to normal tissues to facilitate computation. ARCHITECTURE: 2-Mild reduction in follicle size to 75% of the normal size in at least 25% glandular area; 3-moderate reduction in follicle size to 50% of the normal size in 50% of area; 4-marked reduction in follicle size to 25% or less of normal size in 75% of area. EPITHELIUM: 2-Mild increase in height to moderate cuboidal in 50% of glandular area; 3-moderate increase in height to high cuboidal in 75% of the area; 4-marked increase in height to columnar in 75% of the area. COLLOID: 2-Mild reduction in colloidal density in 50% of glandular area; 3-moderate reduction in the density in 75% of the area; 4-marked reduction in the density in 75% of the area. Results represent mean ± SD obtained from 19 to 20 animals from Group A and 14 to 15 animals from Group B.

[b] Corresponding values from Groups A and B are significantly different ($p < 0.05$).

occurred at 5.0 ppm and higher dose levels. Based on the biochemical, hematological, and histological evaluation data observed in this and previous short-term studies (Chu *et al.*, 1982a, 1984) the no adverse effect level was judged to be 0.5 ppm in the diet (31 μg/kg body wt/day). The highest reported level of OCS in fish was 0.28 ppm (Kuehl *et al.*, 1981) which would give rise to an average daily intake of 4.6 μg/person or 0.078 μg/kg body wt/day (60 kg person) assuming that the average daily intake of fish was 16.6 g/person/day (Statistics Canada, 1983). This means that there is approximately a 400-fold safety factor between potential exposure in humans consuming contaminated fish and the no-effect level observed in the rat.

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